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THE RÔLE OF THE ACTIVITY COEFFICIENT OF THE HYDROGEN ION IN THE HYDROLYSIS OF GELATIN.

By JOHN H. NORTHROP.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 9, 1921.)

I. The Course of the Reaction.

It is generally assumed that enzymes, and catalysts in general, merely accelerate the velocity of a reaction already taking place. In the case of most enzymes the reaction without the enzyme has been carefully studied and is at least as well known as the enzyme reaction. The hydrolysis of proteins without the presence of enzymes, however, has apparently received very little attention, at least from the standpoint of the kinetics of the reaction. It seemed advisable, therefore, in connection with the study of the proteolytic enzymes to secure some data in regard to the spontaneous hydrolysis, especially since the writer had found that the hydrolysis of gelatin by pepsin[1] was very closely connected with the ionization of the protein. It seemed important to determine whether any influence of the ionization could be noticed in the spontaneous reaction. As will be described more fully below, this is found to be the case.

EXPERIMENTAL.

Preparation of the Gelatin.—Gelatin was selected as the protein in these experiments since it had already been used in the study of pepsin hydrolysis and also since it does not precipitate in strong acid and alkali. It is also much more accurately titratable by the formol titration than are the other proteins. The gelatin was prepared from Cooper's powdered gelatin by washing at the isoelectric point as described by Loeb,[2] and was used in a concentration of

[1] Northrop, J. H., *J. Gen. Physiol.*, 1920–21, iii, 211.

[2] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 237.

from 2 to 3 per cent. It was practically salt-free. Several different preparations were made during the course of the work. No difference could be detected in the behavior of these different lots.

The increase in free amino or free carboxyl groups is the most significant quantity as regards the hydrolysis of proteins, since if the accepted views of structure of the proteins are correct, each hydrolytic cleavage results in the liberation of a free amino and a free carboxyl group. Two methods are available for following quantitatively the course of such reaction; Van Slyke's[3] amino nitrogen determination, and Sörensen's[4] formol titration which determines the free carboxyl groups. For absolute determinations of the amino-acids Van Slyke's method is more accurate, for comparative experiments concerning the changes occurring in gelatin solutions, such as were used in this work, the formol titration is more accurate and also much more rapid. Such a slight hydrolysis of gelatin as that required merely to liquefy the protein may be quite accurately determined by the formol titration whereas the increase in amino nitrogen is so small as to be within the limits of error of the Van Slyke method. The formol titration was used therefore in nearly all the experiments reported in this paper. It is well known that many substances, such as phosphates and carbonates, interfere with this titration. In order to avoid these difficulties the method was slightly modified. It is obvious that the final titration figure will depend on the amount of alkali or acid present in the original solution. In order to get comparable results, therefore, it is always necessary to start the titration from the same pH. This was accomplished by titrating the sample to pH 7.0, using neutral red as an indicator, before adding the formaldehyde and continuing with the final titration. In the case of gelatin itself, this method does not give the correct figure since, in order to get the total acidity, it is necessary to start the titration at the isoelectric point of the gelatin; *i.e.*, pH 4.7. The figures obtained by titrating from pH 7.0 are, therefore, too low by the amount of alkali necessary to titrate the gelatin from pH 4.7 to pH 7.0. Since this is a constant quantity for any given concentration of gelatin, the titration figure could be corrected if necessary.

[3] Van Slyke, D. D., *J. Biol. Chem.*, 1913–14, xvi, 121.

[4] Sörensen, S. P. L., *Biochem. Z.*, 1908, vii, 45.

This correction is small, however, and in the case of comparative experiments cancels out. In the titration of the amino-acids the correction is within the limits of error of the titration. A pH of 7.0 was chosen as the standard since the titration curve of gelatin is very flat at this point so that the adjustment of the reaction is accurate to less than 0.05 cc. of 0.1 N NaOH and also since the presence of citrate does not interfere at this pH.

Adjustment of the pH of Gelatin Solutions.—In strongly acid or alkaline solutions it is not necessary to use buffers since the change in the pH during the hydrolysis of the gelatin is neglible. Between pH 1.5 and 11.0, however, it is necessary to use some buffer as otherwise the pH of the solution changes rapidly during the course of the reaction. It was noted above that phosphates and carbonates interfere with the titration. Phosphates are difficult to remove and were not used at all in these experiments. It was found that a complete series of buffer solutions could be prepared with various mixtures of trisodium citrate, HCl, and Na_2CO_3. These solutions were therefore used. Since carbonate also interferes with the titration it was removed by bringing the sample to about pH 2.5 with strong acid, boiling out the CO_2 and then titrating back to pH 7.0. Control experiments showed that this procedure had no effect whatever on the final titration; that is, the figure obtained for 10 cc. of gelatin solution was identical when the gelatin was titrated to pH 7.0 directly before making the formol titration, and when it was titrated first to say pH 10 with Na_2CO_3, then made acid, the carbonate boiled out, and then titrated to pH 7.0. The total concentration of citrate was not above 0.05 N, since more concentrated solutions caused a slight increase in the titration figure and rendered the adjustment to pH 7.0 more difficult.

Formalin Solution.—A solution of formalin was used containing 30 cc. of a saturated aqueous solution of thymol blue per 300 cc. of solution, and titrated with sufficient alkali, so that when 5 cc. were added to 15 cc. of water, the resulting solution had a pH of 8.4. This procedure obviates the necessity of making a correction for the formalin solution. 5 cc. of this solution were used for 10 cc. of the sample.

Example of Titration.—(*a*) *Solution Containing no Carbonate.*—10 cc. of 2.5 per cent gelatin solution or the equivalent amount, if more concentrated gelatin is used, are pipetted into a large test-tube, 1 drop of neutral red is added, and the solution is brought to approximately pH 7.0 by the addition of a few drops of concentrated alkali (carbonate-free) or strong HCl. The reaction is then corrected accurately to pH 7.0 with 0.1 N NaOH (CO_2-free) or 0.1 N HCl, using a standard of pH 7.0 in the comparator block. 5 cc. of the formalin solution are then added and the solution is titrated with 0.1 N NaOH to pH 8.4, using a standard tube with thymol blue for comparison. The neutral red does not interfere with this titration and if anything makes it more sharp. The number of cc. of 0.1 N alkali required to titrate from pH 7.0 to pH 8.4 after the addition of the formalin is noted and is referred to as the formol titration. This value, as Sörensen showed, is independent of the final pH taken, provided this is above 8.2. It represents the number of cc. of 0.1 N acid present in the solution.

(*b*) *Carbonate Present.*—The solution is brought to pH 2.5–3.0 with a few drops of concentrated HCl and boiled for 10 to 20 seconds over the free flame to remove the CO_2. It is then titrated as described under (*a*).

In order to check the accuracy of the method several experiments were made in which the results obtained by the formol titration and by Van Slyke's method were compared. The results of a few of these are given in Table I. The amino nitrogen was determined as described by Van Slyke. The solution was shaken for 30 minutes. In the case of gelatin, the titration figures for gelatin alone, without the addition of formalin are also given. These figures were obtained from the titration curves of gelatin given by Loeb,[5] by correcting for the amount of acid or alkali necessary to bring the same volume of water to the same pH. It is found that below pH 1.8 and above pH 11.0 at 25°C. the figures so obtained are constant and independent of the pH; *i.e.*, outside these limits, the gelatin is all combined as a salt and the figures represent the normality of the solution.

[5] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 85.

TABLE I.

Analysis of Gelatin Solutions by Different Methods.

Solution analyzed.	NH_2N per 2 cc. of solution, Van Slyke method.	Formol titration per 10 cc. of solution.	Equivalent cc. of 0.1 N NaOH calculated to 1 cc. 1 per cent gelatin.		Normality 1 per cent gelatin.
			Van Slyke.	Formol.	
	cc.	cc.	cc.	cc.	
5 per cent gelatin.	0.94	3.00 (direct titration.)	0.036	0.060	0.0036 (Van Slyke).
	0.88	3.05	0.038	0.061	0.0038
		2.05 (titration to pH 7.0. first; neutral red titration.)		0.040	0.0060 (Formol).
		2.03		0.041	
5 per cent gelatin slightly hydrolyzed (just liquefied).	1.00	2.30 "	0.040	0.046	
	1.05	2.25 "		0.045	
2 per cent gelatin partially hydrolyzed.	5.00	11.60 "	0.60	0.58	
	4.80	11.50 "	0.57	0.575	
2.5 per cent gelatin nearly completely hydrolyzed at pH 14.0 containing 0.1 N Na_2CO_3.	10.0	19.5 "	0.80	0.83	
	10.4	20.8 "	0.84	0.78	
1 per cent gelatin titrated directly with HCl or any strong acid.			0.120		0.012
1 per cent gelatin titrated directly with NaOH or Ba(OH).			0.06		0.006

The table shows that the formol titration, if the gelatin is first brought to pH 7.0, gives identical figures with that of the Van Slyke determination at all stages of hydrolysis. It also shows that the direct titration of gelatin without any formalin gives the same value

as that obtained with the formol titration. The formaldehyde evidently merely makes the acid groups stronger so that it is not necessary to titrate to such a high pH.

The figures in the last column show that 1 per cent gelatin solution is about 0.004 normal with respect to free NH_2 groups, 0.006 normal with respect to total acid groups, and 0.012 normal with respect to total basic groups. That is, there must be approximately twice as many basic as carboxyl groups, and three times as many total basic groups as NH_2 groups. These figures agree approximately with those found by Wintgen and Krüger.[6]

Determination of Hydrogen Ion Concentration.—The pH of the solutions was determined before and after hydrolysis. In the case of the buffered solutions no significant change was noted. The determination was made by the E.M.F. method (except in the solutions containing $HgCl_2$) using the rocking electrodes as described by Clark[7] in a constant temperature bath. A saturated KCl calomel electrode was used. The E.M.F. of this electrode was determined before and after every series of determinations against a 1.0 N solution of hydrochloric acid, prepared according to Hulett[8] and checked by conductivity measurements. The pH of this 1.0 N HCl was calculated from the activity coefficient as given by Noyes and MacInnes;[9] *i. e.*, 0.082 at 25°C. This standard was chosen since Fales and Vosburgh[10] have shown that there is no diffusion potential in such a cell and since the strength of the acid can be checked by an independent method; *i.e.*, the conductivity. The value for the saturated KCl electrode found in this way averaged 244 millivolts. It varied about 1 millivolt on either side of this value, from time to time, but was constant within 1 millivolt during the course of any one series of determinations.

Influence of Temperature on the pH.—Since the experiments were carried out at 40°C. and most of the pH determinations were made at 25°C. it was necessary to know what effect this change of tempera-

[6] Wintgen, R., and Krüger, K., *Koll. Z.*, 1921, xxviii, 81.
[7] Clark, W. M., Determination of hydrogen ions, Baltimore, 1920.
[8] Hulett, G. A., and Bonner, W. D., *J. Am. Chem. Soc.*, 1909, xxxi, 390.
[9] Noyes, A. A., and MacInnes, P. A., *J. Am. Chem. Soc.*, 1920, xlii, 239.
[10] Fales, H. A., and Vosburgh, W. C., *J. Am. Chem. Soc.*, 1918, xl, 1291.

ture would have on the pH. In order to determine the value of this temperature effect a series of gelatin solutions containing 2 per cent gelatin and having a pH of from 0.0 to 14.0 was made up. The pH was adjusted by varying amounts of HCl, NaOH, Na_2CO_3, and sodium citrate. The total salt content of all the solutions was between 0.02 and 0.05 N. The pH of these solutions was then determined at 25° and at 37°C. (The electrodes regulated at 37°C. were put at the author's disposal by Dr. Glenn E. Cullen). The results are summarized in Table II. The C_{OH} was calculated by the formula $C_H C_{OH} = K_W$ (or $pH + pOH = \text{Log } K_W$). The value of K_W at 25° was taken as 1×10^{-14} and at 40° as 2.8×10^{-14}. It was found that the hydrogen ion concentration was independent of the tem-

TABLE II.

Effect of Temperature on pH of Gelatin Solution.

pH observed.		pOH calculated.	
25°C.	40°C.	25°C.	40°C.
12.70	12.25	1.30	1.25
12.25	11.75	1.75	1.75
9.92	9.72	4.08	3.78
8.42	8.42	5.58	5.08
5.01	5.02	8.99	8.48
0.76	0.75	13.24	12.75

perature from pH 0.0 to pH 8.8, and that the (calculated) hydroxyl ion concentration was independent of the temperature from pH 11 to pH 14. Between pH 8.8 and 11.0 the concentrations of both hydrogen and hydroxyl ions increased with the temperature. In all the other experiments therefore the hydrogen ion concentration was assumed to remain the same at 25° and 40°C. on the acid side of pH 8.5, and the hydroxyl ion concentration was assumed to remain constant on the alkaline side of pH 11.0. The experiments which fell between pH 8.8 and 11.0 were measured at 37°C.

The Course of the Reaction.—It has been known for a long time that proteins may be hydrolyzed to the amino-acids by prolonged heating with acids or alkali. The kinetics of the reaction, however, have received little or no attention. Since it was desired to compare the

velocity of hydrolysis of the gelatin under different conditions it was first necessary to determine some value to be used as a standard. Two gelatin solutions were therefore prepared, one containing 0.75 N NaOH and the other 4.5 N HCl. These solutions were then placed at 65°C. and the increase in the formol titration followed. The results are shown in Fig. 1. The curves fall off more rapidly than the predicted rate of a monomolecular reaction, as is shown by Fig. 2, in which the logarithm of the quantity of gelatin remaining is plotted against the time, which in the case of a monomolecular

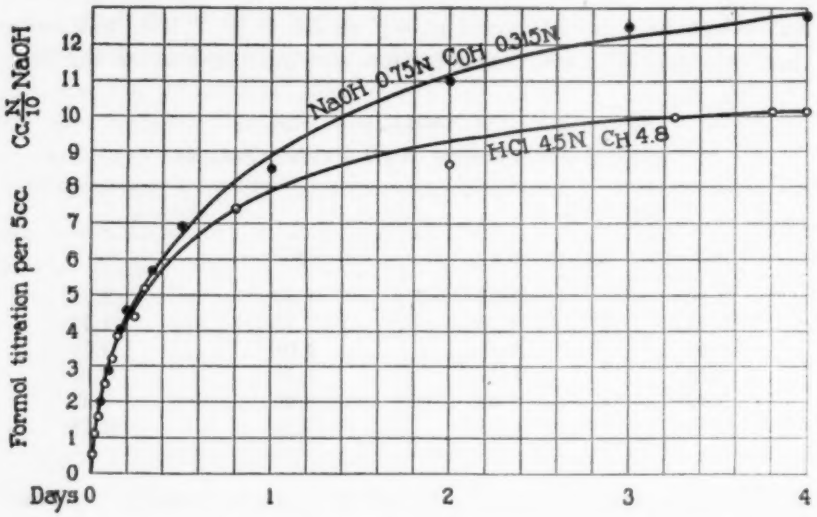

FIG. 1. Increase, with time, in formol titration in strongly alkaline and strongly acid solutions.

reaction gives a straight line. It is obvious from the figure that the reaction is monomolecular for the first 30 or 40 per cent but than becomes too slow. This cannot be ascribed to changes in the C_H or C_{OH} since no measureable change in these values could be detected. This means presumably that the simpler decomposition products are more resistant to hydrolysis than is the gelatin itself. Schroeder[11]

[11] von Schroeder, P., *Z. physik. Chem.*, 1903, xlv, 75. Taylor (Taylor, A. E., *Univ. California Pub., Path.*, 1907, i, 239) states that the hydrolysis of protamine by acid is monomolecular.

found that the rate of decrease of the viscosity of gelatin solutions also follows the monomolecular formula. This would be expected since the decomposition products have a very low viscosity compared to gelatin. It may be mentioned that the final figure reached by the two solutions was nearly identical and agreed with that found by Van Slyke for the total hydrolysis of gelatin. The alkaline solution gave a slightly higher figure due to silicic acid from the glass. Since the gelatin must evidently be destroyed in the very beginning of the reaction, it is this part which must be studied in order to notice any influence of the condition of the gelatin on the velocity of hydrolysis. It will be noticed that the formol titration increases about

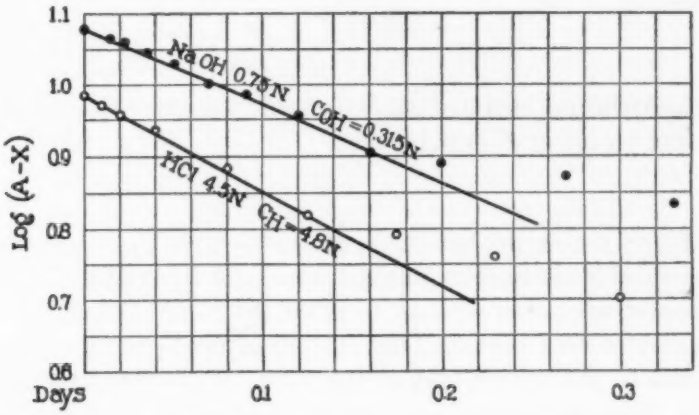

FIG. 2. Increase, with time, in formol titration in strongly aklaline and strongly acid solutions.

20 times during the reaction so that during the first two or three hundred per cent increase in the original figure the increase will be very nearly linear with respect to time.

That this is actually the case is shown in Fig. 3 in which the increase in the formol titration, as expressed in per cent of the original figure is plotted against the time. As the figure shows, the curves of all the experiments in which the hydrogen ion concentration was kept constant are straight lines within the limits of experimental error. Curve 4 in which the hydrogen ion concentration decreased during the hydrolysis, however, drops off quite rapidly. As will be shown

later, the velocity in this range of pH is proportional to the hydrogen ion concentration. Also since the products formed are weak bases we have the condition that[12]

$$R\mathrm{NH_2} + \mathrm{HCl} \rightleftharpoons R\,\mathrm{NH_2.HCl} \qquad (1)$$

and hence, when $R\mathrm{NH_2}$ is large compared to C_H; (assuming complete ionization of the HCl)

$$C_H = \frac{k}{C_{R\mathrm{NH_2}}}$$

i.e., the hydrogen ion concentration and hence the velocity of the reaction will be inversely proportional to the amount of products formed.

$$\text{Velocity} = \frac{dx}{dt} = k\,C_H = \frac{k'}{C_{R\mathrm{NH_2}}} = \frac{k'}{x}$$

This, as Arrhenius[13] pointed out is the condition which causes Schütz's rule since on integration it becomes

$$x = k'' \sqrt{t}$$

In Fig. 4 the results have been plotted against the square root of the time and it will be seen that the experiment in which the hydrogen ion concentration was *not* kept constant (by means of buffers) obeys Schütz's rule very well; *i.e.*, the amount of products formed is proportional to the square root of the time. Reactions in which the rate is inversely proportional to the amount of products formed have been quite frequently observed. A very clear case which was completely worked out and the mechanism verified experimentally is that of the oxidation of oxalic acid by bromine studied by Richards and Stull.[14] Similar reactions are discussed by Müller.[15] The writer

[12] The complete equation would be

$$\begin{array}{rcl} & + \mathrm{HCl} \rightleftharpoons R\mathrm{NH_2} & . \ \mathrm{HCl} \rightleftharpoons R\mathrm{NH_3^+} + \mathrm{Cl^-} \\ R\mathrm{NH_2} & & \downarrow\uparrow \\ & + \mathrm{H_2O} \rightleftharpoons R\mathrm{NH_3OH} & R\mathrm{NH_2^-} + \mathrm{H^+} \end{array}$$

At the range of pH under consideration, however, the equilibrium may be represented approximately by (1).

[13] Arrhenius, S., *Medd. Kong. vetsakad. Nobelinst.*, 1908, i.

[14] Richards, T. W., and Stull, W. N., *Z. physik. Chem.*, 1902, xli, 544.

[15] Müller, W., *Z. physik. Chem.*, 1902, xli, 483.

was able to show[16] that in pepsin digestion the same mechanism causes the reaction to follow the square root law since the pepsin and the products of digestion are in equilibrium. The pepsin in this case replaces the hydrogen ion in the present example.

Returning to the experiments in which the hydrogen ion concentration was kept constant it will be seen that the rate of reaction is practically constant for at least the first 400 per cent increase in the original titration (equivalent to the first 20 per cent of the reac-

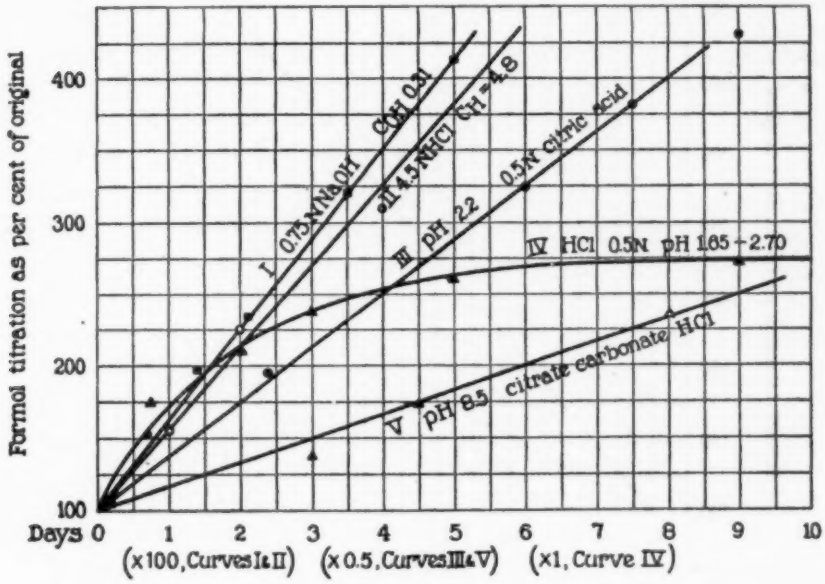
Formol titration as per cent of original
400
300
200
100
Days 0 1 2 3 4 5 6 7 8 9 10
(x100, Curves I & II) (x 0.5, Curves III & V) (x1, Curve IV)
I 0.75 N NaOH
II pH 4.3 NH4Cl CH = 4.8
III pH 2.2
IV HCl 0.5N pH 1.65 - 2.70
V pH 8.5 citrate carbonate HCl
0.5N citric acid

FIG. 3. Course of reaction with various hydrogen ion concentrations.

tion). The velocity within this range may, therefore, be determined at any time by dividing the increase in the titration by the elapsed time. If the increase is expressed as per cent of the original figure this ratio will represent the velocity of the reaction and will be independent of the concentration of gelatin. It seems more convenient and equally significant for the purposes of this paper to use this figure rather than the constant for the monomolecular reac-

[16] Northrop, J. H., *J. Gen. Physiol.*, 1919–20, ii, 471.

tion. The velocity will therefore be considered as the increase expressed as per cent of the original titration divided by the elapsed time in days. The resulting figure is the per cent increase in the original titration per day, assuming that the amount of unhydrolyzed gelatin remains approximately unchanged. In most of the experiments determinations were made at several different times and the velocities so obtained were averaged. In all cases the experiment

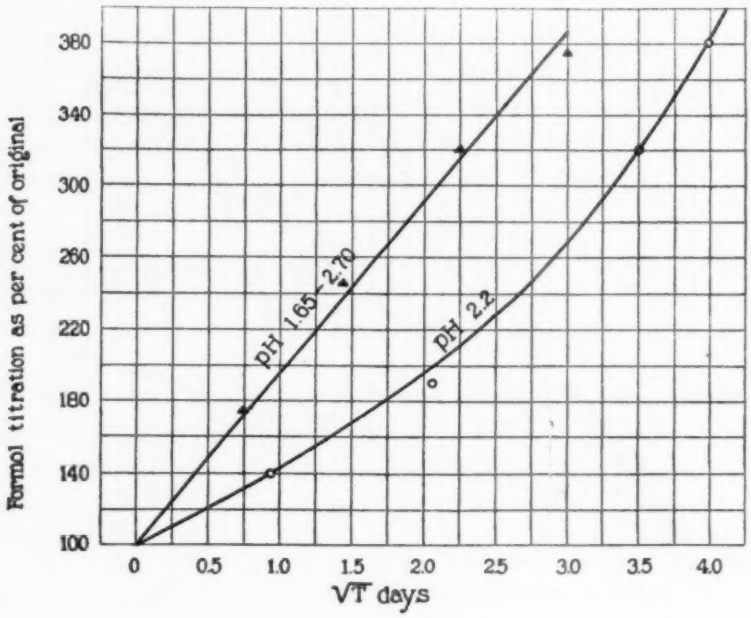

FIG. 4. Comparison of the course of reaction with constant and with varying hydrogen ion concentrations.

was continued until the original titration had increased at least 50 per cent and in all, except a few between pH 5.0 and 7.0, until the titration value had increased over 200 per cent.

Concentration of Gelatin.—Since the reaction approximates that of a monomolecular, the velocity when expressed as percentage change should be independent of the gelatin concentration. Table III contains the result of an experiment in which the gelatin concentration was varied from 10 to 1.25 per cent; it shows that the velocity is independent of the concentration for the early stages of the hydrolysis.

TABLE III.

Influence of Concentration of Gelatin at 65°C.
Gelatin Per Cent Concentration Noted + HCl to pH 2.0.

Concentration.	C_H	Velocity = $\frac{\text{per cent change}}{\text{time in days}}$.
per cent		
10	2.00	16
5	2.08	16, 14
2.5	2.03	17
1.25	2.02	17, 18

II. The Influence of High Concentrations of Hydrogen and Hydroxyl Ions.

It was shown above that the rate of the reaction decreased very rapidly if the hydrogen ion concentration was allowed to decrease. This in itself indicates that the rate is a function of the hydrogen ion concentration and not of the total amount of acid present. The fact that it is the hydrogen ion concentration and not the total acidity

TABLE IV.

Influence of Different Acids on Velocity of Hydrolysis.
2.5 Per Cent Gelatin at 65°C. with pH Measured at 25°C.

Acid.	Concentration.	C_H 25° × 10^3.	Velocity.	$\frac{\text{Velocity}}{C_H} \times 10^{-3}$.
	N			
HCl	0.024	6.0	18	3.0
HNO_3	0.024	6.0	17	2.8
H_2SO_4	0.024	6.3	16	2.5
Oxalic	0.050	6.3	16	2.5
Citric	0.33	5.6	16	2.8
		5.1	15	2.9

is shown more clearly in Table IV which contains the results of an experiment in which the gelatin was brought to the same pH with different acids. The table shows that the velocity is the same in all the solutions although the total concentration of citric acid was more than 10 times that of the HCl.

In all the above experiments the hydrogen ion concentration has been expressed as that calculated by Nernst's formula from the potential of the hydrogen electrode. It is certain that this value does not really express the actual concentration in grams per liter of hydrogen ions. It would probably be better to call it the "activity coefficient" or "active concentration" as proposed by Lewis;[17] that is, it represents that value which must be assigned to the hydrogen ion concentration in order to have it obey the law of mass action as assumed in Nernst's equation. In low concentration, however, the value so obtained does not differ very much from that obtained by the conductivity method. In more concentrated solution the discrepancy becomes greater and may amount to several hundred per cent in concentrated HCl. It seemed of interest to determine which of these values is the significant one for the hydrolysis of gelatin. A series of experiments was therefore made in which the concentration of acid was varied from 1.0 N to about 4.1 N acid. The results are shown in Table V. The total acidity was determined approximately by titration. The values for the hydrogen ion concentration by the conductivity measurements were interpolated from Kohlrausch's tables. The hydrogen ion concentrations as determined by the E. M. F. method agree fairly well with those given by Noyes and MacInnes[9] except in the case of the strongest HCl concentration in which it is too low. It is remarkable that the rate of hydrolysis is in all cases nearly proportional to the hydrogen ion concentration as determined from the E. M. F. measurements. It is clear therefore that it cannot be the concentration of hydrogen ions as expressed in grams per liter which determines the rate of the reaction, since in the case of 3.7 N HCl it would be necessary to assume nearly 120 per cent dissociation. On the other hand, the result cannot be ascribed to the undissociated acid since it is hardly conceivable that the undissociated acid could effect the hydrogen electrode. It seems necessary to conclude that it is the "activity" of the hydrogen ion which determines the rate of hydrolysis of gelatin as well as the potential of the hydrogen electrode.

[17] Lewis, G. N., *Proc. Am. Acad. Arts and Sc.*, 1907, xliii, 259.

In other words, the "concentration" as determined by the hydrogen electrode is the effective concentration for the present reaction.

In a very recent paper Schreiner[18] has suggested the use of a special "catalysis-coefficient" to express the relation of the apparent hydrogen ion concentration, as determined by catalysis experiments, to the total acid concentration. Schreiner states that this coefficient is equal to the reciprocal of the conductivity ratio (corrected for

TABLE V.

Hydrolysis of 2.5 Per Cent Gelatin in Strong Acid at 40°C.

Acid.	Approximate concentration.	C_H E.M.F.	C_H Conductivity.	Velocity.	$K_H = \frac{\text{Velocity}}{C_H \text{ (E.M.F.)}}$	$\frac{\text{Velocity}}{C_H \text{ (conductivity)}}$
	N					
HCl	4.1	[5.5]	1.97	800	[130]	400
HCl	3.7	5.4	1.88	790	146	400
H_2SO_4	3.7	2.0	1.57	300	151	200
HCl	3.1	3.6	1.75	600	167	340
H_2SO_4	3.0	1.6	1.40	180	[125]	130
HCl	2.2	2.7	1.48	380	140	250
HCl	2.1	2.10	1.40	[400]	[190]	[280]
H_2SO_4	2.0	0.90	1.00	130	146	130
HCl	1.5	1.50	1.06	220	140	210
HCl	1.0	0.84	0.80	142	170	178
HCl	1.0	0.85	0.80	130	160	162
HCl	1.0	0.89	0.80	[160]	[180]	[200]
H_2SO_4	1.0	0.40	0.50	60	150	120
HCl	0.7	0.72	0.60	110	155	185
K_H = average					152	

viscosity), and he is able by its use to calculate quite closely the velocity of hydrolysis of methyl and ethyl acetate in concentrated acid or acid-salt mixtures. It seems unnecessary to introduce such a coefficient in order to account for the results of the experiments reported here, since it is possible to calculate the velocity, within the rather large experimental error, by means of the activity coefficient alone.

[18] Schreiner, E., *Z. anorg. u. allg. Chem.*, 1921, cxvi, 102.

III. Effect of Neutral Salts.

It was noted by Arrhenius[19] in 1899 that the addition of neutral salts to a weak acid increased the rate of hydrolysis of methyl acetate by the acid, which is just the opposite of the effect expected from the law of mass action. Arrhenius originally assumed that the salt actually increased the concentration of hydrogen ions. This view was criticised, however, and the hypothesis was put forward independently[20] by Dawson, and by Senter and Acree that the observed effect was due to the undissociated acid and a large number of experiments were performed which strengthened this view. It was found by Nelson and Fales,[21] however, that under certain conditions the effect of neutral salts on the activity of invertase could be ascribed entirely to the effect of the salt on the hydrogen ion concentration as measured by the hydrogen electrode. Since that time evidence has accumulated to show that the addition of neutral salts to a strong acid causes an increase in the hydrogen ion concentration as measured by the hydrogen electrode. A series of experiments was therefore performed to determine whether the addition of salts would influence the hydrolysis of gelatin. The results of this series are given in Table VI. It will be seen that the addition of 1.5 N NaCl to 1.0 N HCl solution increases the hydrogen ion concentration by nearly 50 per cent and that the rate of hydrolysis is increased practically the same amount. That is, if the effect of the neutral salt on the hydrogen ion concentration is taken into account, there is no effect on the rate of hydrolysis. In the case of NaCl and $CaCl_2$ the result is the same up to 1.0 N but above that the hydrogen ion concentration is increased more than the rate of hydrolysis. It was thought at first that this might be due to diffusion potentials caused by the NaCl or $CaCl_2$, since it will be noted that NaCl and $CaCl_2$ increase the hydrogen ion concentration more than the same concentration of KCl, whereas the effect on the rate of hydrolysis is the same for all the salts. (This is the result obtained

[19] Arrhenius, S., *Z. physik. Chem.*, 1899, xxxi, 197.

[20] For a review of this question see Lewis, W. C. McC., A system of physical chemistry, London, 1918–19, i.

[21] Fales, H. A., and Nelson, J. M., *J. Am. Chem. Soc.*, 1915, xxxvii, 2769.

TABLE VI.

Effect of Addition of Salts on Hydrolysis of 2.5 Per Cent Gelatin at 40°C. HCl Concentration 1.0 N.

Salt.	Concentration.	pH	C_H E.M.F.	Velocity.	$K_H = \frac{\text{Velocity.}}{C_H}$
	N				
0	0	0.07	0.85	142	168
0	0	0.08	0.83	140	169
KCl	1.0	+0.06	1.15	176	153
KCl	1.0	+0.05	1.12	177	158
KCl	1.5	+0.08	1.20	182	151
KCl	1.5	+0.08	1.20	185	154
0	0	0.07	0.84	145	172
NaCl	0.5	+0.17	1.04	170	163
NaCl	1.0	+0.09	1.23	180 190	146 155
NaCl	1.5	+0.17	1.48	180	120
NaCl	1.65	+0.20	1.58	187	118
NaCl	1.65	+0.20	1.58	172	109
NaCl	1.65	+0.24	1.74	174	100
$CaCl_2$	0.5	+0.034	1.08	162	150
$CaCl_2$	1.0	+0.08	1.20	178	148
$CaCl_2$	1.5	+0.23	1.70	185	110
65°C. HCl Concentration 0.02 N.					
0	0		0.0049	10	2.0×10^3
0	0		0.0045	11	2.4×10^3
KCl	0.5		0.0043	10	2.3×10^3
KCl	0.5		0.0043	13	3.0×10^3
KCl	1.0		0.0043	12	2.8×10^3
KCl	1.0		0.0045	10	2.2×10^3
KCl	1.5		0.0041	12	2.9×10^3
KCl	1.5		0.0041	12	2.9×10^3

by Arrhenius also.) Measurement showed, however, that there was no diffusion potential in the case of NaCl at least. The diffusion potential was measured by determining the E. M. F. of the hydrogen electrode immersed in the NaCl-HCl solution, first against the sat-

urated KCl calomel electrode (in which case the hydrogen electrode in the NaCl-HCl is negative), and second, against another hydrogen electrode immersed in 1.0 N acid; a saturated KCl bridge was used in both cases. In this case the electrode in the NaCl solution is positive. Any diffusion potential therefore would tend to increase the measured potential in one case and decrease it in the other, so that the potential between the two hydrogen electrodes when measured directly should differ from the difference in potential of the two measured against the same calomel electrode by twice the value of the diffusion potential. The experiment showed, however, that an identical value was obtained by either method.

The experiments summarized in Table VI show that the effect of neutral salts can be accounted for by the increase of the hydrogen ion concentration. Whatever discrepancy is found is due to the fact that the hydrogen ion concentration apparently increased more than the rate of hydrolysis. It is evidently unnecessary to assume any activity of the unionized acid in this case at least.

Experiments in Low Concentration of Acid.—The above experiments were repeated with 0.02 N HCl (at 65°C.). In this case the addition of KCl has a very slight depressing effect on the hydrogen ion concentration and little if any influence on the rate of hydrolysis. This experiment also shows that the unionized gelatin chloride must hydrolyze at approximately the same rate as the ionized. It was shown by conductivity measurements[1] that gelatin chloride is practically completely ionized at pH 2.4, and that the addition of 1.0 N Cl ion reduces the ionization to a very small amount. It also decreases the rate of hydrolysis by pepsin very markedly and in direct proportion to the decrease in the conductivity of the gelatin chloride. The hydrolysis of gelatin by acid and by pepsin differs markedly in this respect. In pepsin hydrolysis the rate of digestion is decreased by increasing the amount of acid or salt beyond 0.01 N whereas in the acid hydrolysis the rate is unaffected by the addition of salt and increases in direct proportion to the hydrogen ion concentration with the addition of more acid. These facts agree with the hypothesis that the rate of hydrolysis of the ionized and non-ionized gelatin salt by hydrogen ions is the same, but that the ionized gelatin salt hydrolyzes much more rapidly than the non-ionized in the presence of pepsin.

IV. Influence of the Hydroxyl Ion Concentration.

It has been shown above that the velocity of hydrolysis of gelatin is directly proportional to the hydrogen ion concentration as measured

TABLE VII.

Hydrolysis of 2.5 Per Cent Gelatin in Strong Alkali at 40°C.

Alkali.	Concentration.	$C_{OH} = \frac{10^{-13.5}}{C_H}$.	Velocity.	$K_{OH} = \frac{\text{Velocity}}{C_{OH}\ (\text{E.M.F.})\ 25°C.}$
	N			
$Ba(OH)_2$.....	0.16	0.05	250	5,000
KOH.....	0.140	0.047	225	4,800
NaOH.....	0.12	0.045	230	5,100
KOH.....	0.69	0.30	1,000	3,300
NaOH.....	0.78	0.30	1,300	4,330
NaOH.....	1.0	0.45	2,250	5,000
KOH.....	1.27	0.60	2,300	3,900
NaOH.....	1.36	0.63	3,300	5,300
KOH.....	1.91	1.25	5,000	4,000
NaOH.....	2.0	0.90	6,000	6,600
NaOH.....	2.04	0.91	[8,000 9,000]	[9,000 10,000]
NaOH.....	2.72	1.20	10,000	[8,400 7,100]
NaOH.....	3.0	1.26	9,000	
KOH.....	2.55	2.0	8,500	4,250
KOH.....	3.82	3.2	17,000	5,300
NaOH.....	4.08	1.78	20,000	[11,000 7,500]
NaOH.....	4.1	1.86	14,000	
NaOH.....	4.9	2.70	21,000	7,800
Average.....				4,700

by the hydrogen electrode in acid concentrations of from 0.10 to 4.1 N. In Table VII the results of a series of determinations in strong alkali are given. The concentration of hydroxyl ions was calculated

from the pH, taking the value for the dissociation constant of water at 40°C. as 2.8×10^{-14}. The rate is directly proportional in this range to the hydroxyl ion concentration as determined by the hydrogen electrode. It is not possible to compare the hydroxyl ion concentration as determined by the conductivity since the alkali solutions contained some carbonate. The constant is not so good here as in the acid solution since the reaction in strong alkali is extremely rapid. It will be noticed that the proportionality factor for the hydroxyl ion is 4700 whereas that for the hydrogen ion was 150; *i.e.*, the hydroxyl ions hydrolyze about 30 times more rapidly than hydrogen ions at the same concentration. It is not possible to say whether this difference is due to the hydrogen and hydroxyl ions or to the difference in the ease of hydrolysis of the gelatin-acid salt and the alkali gelatin.

V. Influence of Hydrogen and Hydroxyl Ions in Low Concentration.

It has been shown above that the velocity of hydrolysis in the acid range may be calculated from the equation

$$\text{Velocity}_{H} = 150\, C_{H}$$

and in strongly alkaline solutions from the equation

$$\text{Velocity}_{OH} = 4700\, C_{OH}$$

Combining these two equations we would calculate that (in round numbers) the velocity at any $C_H = 150\ (C_H + 30\ C_{OH})$. This is the same type of equation found by Wijs[22] to represent the influence of the concentration of hydrogen and hydroxyl ions on the rate of hydrolysis of methyl acetate. The formula predicts that the rate of hydrolysis is a minimum when the $C_H = 30\ C_{OH}$ or at 40°C. at about pH 6.0. (The mathematical proof of this statement is that the first derivative of the above expression is zero at the point where $C_H = 30\ C_{OH}$, and would be equal to approximately 3×10^{-4}; *i.e.*, it would take nearly 30 years to cause an increase of 3 per cent in the formol titration of a gelatin solution at 40°C.) It was found, however, that the hydrolysis actually occurred at pH 6.0, about

[22] Wijs, J. J. A., *Z. physik. Chem.*, 1893, xii, 514.

300 times more rapidly than that predicted by the formula. The results of a series of experiments covering the range of from pH 1.0 to pH 12 are given in Table VIII and Fig. 5. In this figure the black circles are the experimental points; the line, the graph of the expression, Velocity = 150 (C_H + 30 C_{OH}); and the circles, the rate calculated from a formula considered below. It is necessary in this range to use some precaution to prevent the growth of microorganisms. Several different substances were used as indicated in the table. Control experiments were made at 65°C. on the influence of these

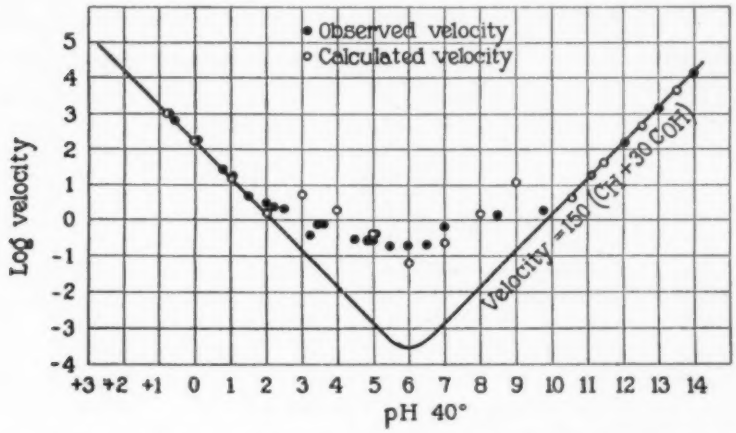

FIG. 5. Influence of the hydrogen ion concentration on the velocity of hydrolysis at 40°C.

on the rate of reaction—no significant effect was noted. In the case of $HgCl_2$ the pH was determined electrometrically before adding the $HgCl_2$ and then colorimetrically. It is a curious fact that nearly all disinfectants poison the hydrogen electrode. The experiment shows that the velocity of hydrolysis is directly proportional to the hydrogen ion concentration from pH 0.65 to pH 2.0. It then decreases much more slowly than the hydrogen ion concentration, passes through a very flat minimum at about pH 6.0 and then increases to become directly proportional to the hydroxyl ion concentration at about pH 10.0. The position of the minimum cannot be told with certainty from the figures given, since the difference

TABLE VIII.

Buffer.	Preservative.	40°C.	40°C.	Velocity.	$K_1 = \frac{\text{Velocity}}{C_H + 30\,C_{OH}}$.	$K_1 = \frac{\text{Velocity}}{(C_H + 30\,C_{OH})\,(C_{uncombined} + 200\,C_{combined})}$.
		p_H	C_H			
See Table V.....		+0.74	5.5 to 1.0	800–100	130–150	130–150
HCl.....		0	1	165	165	165
HCl.....		0.76	0.17	28	165	165
HCl.....		1.0	0.10	15	150	150
HCl.....		1.57	0.03	5	166	160
HCl.....	Toluene	1.80–1.9	1.2, 1.6 $\times 10^{-2}$	3.0	180, 250	180
Citric.....	Toluene	2.20	6.3×10^{-3}	2.8	440	350
HCl citrate.....	Benzoate	2.54	2.9×10^{-3}	2.4	830	30
Citrate-HCl.....	$HgCl_2$	3.17	6.8×10^{-4}	0.4	600	150
Citrate-HCl.....	Benzoate	3.60	2.5×10^{-4}	0.8	3,200	15
HCl citrate.....	$HgCl_2$	4.50	3.1×10^{-5}	0.3	10,000	60
	$HgCl_2$	4.90	1.2×10^{-5}	0.3	21,000	120
Citrate HCl.....	$HgCl_2$	4.90	1.2×10^{-5}	0.3	26,000	120
HCl.....	Toluene	4.9	1.2×10^{-5}	0.3	25,000	
	Toluene	5.01	1×10^{-5}	0.5	50,000	225
Citrate.....	$HgCl_2$	5.4	4×10^{-6}	0.1, 0.2	31,200	150
Citrate.....	$HgCl_2$	6.0	1×10^{-6}		100,000	700
Citrate.....	$HgCl_2$	6.5	3.1×10^{-7}	0.2	64,000	450
			C_{OH} 40°C.			
Citrate-Na_2CO_3....	Benzoate	7.2	5×10^{-7}	0.2	12,000	90
	Benzoate	8.7–8.4	$1.6\text{–}0.8 \times 10^{-5}$	1.8	3,700	150
					1,850	75
Na_2CO_3.....	Thymol Toluene	9.75	1.8×10^{-4}	2.0	370	
NaOH, Na_2CO_3...		11.1	4×10^{-3}	18	150	150
NaOH, Na_2CO_3....	Benzoate	11.1	4×10^{-3}	20	165	165
NaOH.....		12.20	5×10^{-2}	250	165	165
NaOH.....		12.50	0.10	330	110	110
See Table VII....			0.05–3.2	250–1,700	160 ± 10	160

in the rates is not much greater than the experimental error. The determination of the minimum was checked, however, by noting the degree of liquefaction of the solutions after 90 days at 40°C. 10 cc. of the various solutions were brought to pH 5.0, the salt concentration and total volume made the same in all the tubes, and the tubes then immersed in a water bath at 5° for 2 hours. The degree of liquefaction was then noted. The result is given in Table IX. According to this determination the minimum point is about pH 6.5.

Two hypotheses may be suggested to explain this anomalous influence of low concentrations of hydrogen and hydroxyl ions. First, the velocity of hydrolysis is independent of the hydrogen ion concentration between pH 2.0 and 11.0 (or is proportional to the product of $C_H \times C_{OH}$). Second, some change takes place in the gelatin solution

TABLE IX.

pH	7.2	6.5	6.0	5.3	4.8
Liquefaction after 2 hours, 5°C.	+	±	+	+++	++++

in this range which causes the gelatin to hydrolyze very much more rapidly and so compensate for the decrease in the C_H and C_{HO}.

It seems very unlikely from our knowledge of reactions in general that the rate of a reaction should be quantitatively proportional to the hydrogen ion concentration over a wide range and then suddenly become independent of it, to become later directly proportional to the hydroxyl ion concentration.

If we assume temporarily that the rate really is proportional to the hydrogen and hydroxyl ion concentration and that the discrepancy between the observed rate and the rate calculated on this basis is due to a change in the gelatin, we must assume that the gelatin is changed to a form which is very much more rapidly hydrolyzed. The value of the expression $\frac{\textit{observed velocity}}{C_H + 30\ C_{OH}}$ will evidently be a measure of this change. In Fig. 6 the logarithms of the values of this expression are plotted over the pH together with the titration

curve of gelatin (taken from the titration curve for gelatin determined by Loeb), expressed as the per cent of uncombined gelatin present. It is apparent that the two curves are strikingly similar. Both are parallel to the X axis below pH 2 and then rise to a maximum, one at pH 5.0 and one at pH 6.0. Both then drop rapidly and again become parallel to the X axis beyond pH 11.0. This renders it probable that the rate of hydrolysis is some function of the amount of uncombined gelatin present. It will be noted from the table that the value of the "constant" at pH 5.0 (where the gelatin is completely uncombined) is about 200 times that of its value in the range where the gelatin is all present in the form of salt. If we assume then as a first approximation that the uncombined gelatin

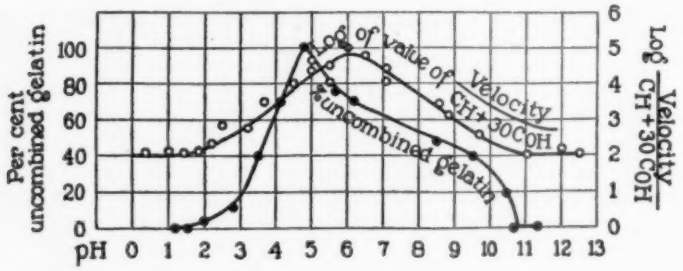

FIG. 6. Influence of the hydrogen ion concentration on percentage of uncombined gelatin and on the value of $K_1 = \frac{\text{elocity}}{C_H + 30C_{OH}}$.

hydrolyzes 200 times as rapidly as the combined and that the relative efficiency of the H and OH ions remains the same we get for the velocity of the reaction

$$\text{Velocity} = K_2 (C_H + 30\ C_{OH})\ (C_{combined} + 200\ C_{free}) \qquad (2)$$

where $C_{combined}$ and C_{free} refer to the concentration of free and combined gelatin as determined from the titration curve. The sum of these two quantities is taken as 1. The values for K_2 are given in the last column (Table VIII). It is evident that although they can hardly be considered as constant they are very much more so than the values for K_1. The rates calculated from (2) are plotted in Fig. 5. They may be considered satisfactory as a rough approximation.

It must be remembered that a slight error in the pH determinations causes a very large variation in the constant and that a shift of the steeper portions of the titration curves of less than 0.2 pH will make a difference in the value of K_2 of several hundred per cent. It must also be noted that the values on the figure are plotted as the logarithms so that the discrepancies are larger than they appear.

It may be pointed out that a reaction very similar to the above was studied by Clibbins and Francis[23] in connection with the hydrolysis of nitrosotriacetoneamine. In this case also the reaction is directly proportional to the C_{OH} over a wide range and then becomes nearly independent of it. In still more alkaline solution the velocity becomes inversely proportional to the C_{OH}. Clibbens and Francis consider also that this is due to some change in the condition of the substance undergoing the reaction but were unable to account for it quantitatively. Many reactions have been studied in which the velocity of reaction of the ions was very different from that of the free substance. Richards and Stull[14] found that, in the reaction between oxalic acid and bromine, the divalent oxalate ion reacts very much more rapidly than any of the other forms present. Stieglitz[24] accounted for the mechanism of the hydrolysis of the imido-esters by the hypothesis that the ester-salt hydrolyzed either more or less rapidly than the free ester. Similar explanations have been proposed by Acree, Goldschmidt, and others.

It will be noted that the peculiar results obtained in the present work are all in the range of acidity where the enzymes are active and where the growth of microorganisms is possible. It seemed possible, therefore, that the increased rate in this range might be due to traces of enzymes or to the presence of bacteria or the action of the various preservatives. The experiments were therefore repeated at a temperature of 65°, without the addition of preservatives. Any traces of enzymes present would be inactivated very rapidly at this temperature and their effect would be noticed only for the first few hours at most. No such effect was noted. The

[23] Clibbens, D. A., and Francis, F., *J. Chem. Soc.*, Tr. 1912, ci, 2358. Francis, F., and Geake, F. H., *Soc., J. Chem.* Tr. 1913, ciii, 1722.

[24] Stieglitz, J., *Am. Chem. J.*, 1908, xxxix, 29.

results of this series are given in Fig. 7. The pH measurements were made at 25° and calculated to 65° assuming the constant for water as 10^{-13} at this temperature and that the hydrogen ion concentration remains constant up to pH 8.0, and the hydroxyl ion concentration constant above pH 11.0. The curve is similar in every respect to that for 40°. There seems no possibility, therefore, that the anomalous rate of hydrolysis between pH 2.0 and 10.0 can be due to enzymes or microorganisms.

It will be noted that the rate of hydrolysis is 10 times more rapid at 65° than at 40° if solutions having the same hydrogen ion con-

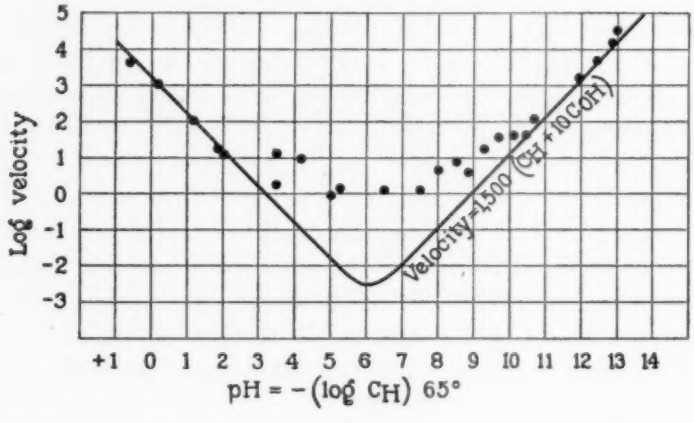

FIG. 7. Influence of the hydrogen ion concentration on the velocity of hydrolysis at 65°C.

centration at both temperatures are compared, but only 3 times more rapid if solutions having the same hydroxyl ion concentration are compared. This latter value seems remarkably low. A similar peculiarity was noticed by Michaelis and Rothstein[25] in studying the influence of the C_{OH} on the destruction of pepsin. These authors found that the destruction of pepsin was proportional to the third power of the C_{OH}, and that, if this was taken into account, the temperature had no effect on the velocity of the reaction.

Mechanism of the Reaction.—It was mentioned in the beginning of the paper that the hydrogen ion concentration decreases rapidly

[25] Michaelis, L., and Rothstein, W., *Biochem. Z.*, 1920, cv, 60.

unless some means are provided such as buffer solutions for keeping it constant. The same is true for the hydroxyl ion concentration. According to the classical definition, therefore, the reaction is not catalytic since some of the catalyst combines with the products of the reaction and so would effect the final equilibrium. It seems probable that this is always true to some extent and that as was emphasized by Stieglitz, a "catalytic" reaction is merely the limiting case of an ordinary reaction in which the products of the reaction dissociate more or less completely liberating more or less of one of the original substances (cf. also Lewis[26] and Falk[27]). If the dissociation is complete no change could be detected in the concentration of one of the reacting substances and the reaction would be monomolecular in regard to the other. If there were no dissociation the reaction would, of course, be bimolecular. The present reaction is intermediate between the two. It is exactly analogous in this respect to the hydrolysis of gelatin by pepsin.

SUMMARY.

1. The hydrolysis of gelatin at a constant hydrogen ion concentration follows the course of a monomolecular reaction for about one-third of the reaction.

2. If the hydrogen ion concentration is not kept constant the amount of hydrolysis in certain ranges of acidity is proportional to the square root of the time (Schütz's rule).

3. The velocity of hydrolysis in strongly acid solution (pH less than 2.0) is directly proportional to the hydrogen ion concentration as determined by the hydrogen electrode *i.e.*, the "activity;" it is not proportional to the hydrogen ion concentration as determined by the conductivity ratio.

4. The addition of neutral salts increases the velocity of hydrolysis and the hydrogen ion concentration (as determined by the hydrogen electrode) to approximately the same extent.

[26] Lewis, W. C., McC., A system of physical chemistry, 2nd ed., London, New York, Bombay, Calcutta, Madras, 1918–19, i, 416.

[27] Falk, K. G., The chemistry of enzyme actions, American Chemical Society Monograph Series, New York, 1921, 33.

5. The velocity in strongly alkaline solutions (pH greater than 10) is directly proportional to the hydroxyl ion concentration.

6. Between pH 2.0 and pH 10.0 the rate of hydrolysis is approximately constant and very much greater than would be calculated from the hydrogen and hydroxyl ion concentration. This may be roughly accounted for by the assumption that the uncombined gelatin hydrolyzes much more rapidly than the gelatin salt.

ENERGY AND VISION.

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(Received for publication, April 19, 1921.)

Although a large number of papers and books have been published on the problems of vision (1), a very limited amount of work is to be found on the minimum energy necessary to produce visual sensation. The classical work of Langley (2) for different wave lengths the papers of Grijns and Noyons (3), Zwaardemaker (4), and Kries (5), for white light are always quoted, but the figures given by different authors do not always agree, discrepancies of 100 per cent, sometimes of 1000 per cent, being frequent, with no explanation. For this reason, it was thought advisable to check all these figures, in order to ascertain whence came the discrepancies. Furthermore, as Langley's figures are given by himself with a certain degree of approximation, and were calculated for the light emitted by the sun, we thought it would be interesting to check them by another method, for another source of light, the Nernst lamp, for instance. These are the reasons for carrying on this series of measurements.

In order to give an idea of how difficult it is to find a figure corresponding to the *minimum visibile* for a certain wave length, we will give an example. Langley's figures are quoted as follows for the wave length 0.55μ, for which the human eye shows a maximum of sensitivity:

By Broca (6)..... 5.6×10^{-9} ergs
By Henri and des Bancels (7)..... 3.0×10^{-8} ergs

whereas Langley's *real* figure, as given in his paper, is 2.8×10^{-9} ergs. Furthermore, Henri and des Bancels state on another page that 10^{-10} is the order of magnitude of the minimum energy necessary to produce the sensation of vision in the green (0.55μ), and Langley (2)[1] states that it is 1.0×10^{-8} (for practically the same radiation, 0.53μ). In order to clear this matter up, we have to go over Langley's paper carefully. Langley states and gives a solution for two different problems: first, determination of the intensity of light necessary to read a table

[1] Langley, (2), p. 23.

of logarithms or to discern any arbitrary character and second, determination of the *minimum visibile*; namely, the minimum of energy which can produce the sensation of light on the retina. The results of the first determinations are expressed in tables (2)[2] in function of the wave length and of the sensitiveness of the eye, in arbitrary figures related to the apparatus and inversely proportional to the energy. The results of the second determinations are expressed, on the contrary (2)[3] in the following way: reciprocals of calories = reciprocals of ergs (let us call this Table A), and these values stated in terms of horse-power (Table B). Now the meaning of these tables is quite ambiguous, and it is not surprising that authors have been mistaken in quoting them because, as they are given, they are only consistent provided the first table (A) is given in ergs per $\frac{1}{2}$ second. But as the figures in Table B, being expressed in horse-power, cannot be given in $\frac{1}{2}$ seconds (as the horse-power unit carries in itself its time unit and can only be used in connection with it, namely, 1 second), the figures of Table A must first be transformed into ergs per second, that is, multiplied by 2, to be identical with those of Table B. This is what has probably escaped the attention of Broca, and of Henri and des Bancels, and unfortunately, Broca took his figures from Table B, and Henri and des Bancels took theirs from Table A, so that all the figures of Broca are exactly double those of Henri and des Bancels. It is possible that these authors have not been mistaken, and that one of them (Broca) reduced the figures in Table A to ergs per second, whereas Henri and his coworker simply took them as they were. But it is most regrettable that none of them gave any indication as to the unit of time. Moreover, an important error is to be found in the figures of Henri and des Bancels due perhaps to misprint: for the wave length 0.55μ, they quote 3.0×10^{-8} ergs, (7)[4] instead of exactly 2.77×10^{-9}. If 3 may be taken as a roughly rounded figure for 2.77, however, the order of magnitude is different. In Broca's quotation, another error or misprint is also to be found: 3.6×10^{-3} ergs for 0.75μ, instead of 2.56×10^{-3}.

It may be of interest to compare the tables published by Broca, and Henri and des Bancels with the exact figures of Langley:

TABLE I.

	Langley.			Henriand des Bancels.	Broca.
	Reciprocal of				
				ergs	*ergs*
0.40μ	1,500,000 $\frac{\text{ergs}}{\frac{1}{2}\text{ sec.}}$	$= 6.7 \times 10^{-7}$ $\frac{\text{ergs}}{\frac{1}{2}\text{ sec.}}$	$= 1.33 \times 10^{-6}$ $\frac{\text{ergs}}{\text{sec.}}$	6.7×10^{-7}	1.37×10^{-6}
0.55μ	360,000,000 "	$= 2.77 \times 10^{-9}$ "	$= 5.55 \times 10^{-9}$ "	3.0×10^{-8}	5.6×10^{-9}
0.65μ	1,600,000 "	$= 6.29 \times 10^{-7}$ "	$= 1.26 \times 10^{-6}$ "	6.0×10^{-7}	1.26×10^{-6}
0.75μ	780 "	$= 1.23 \times 10^{-3}$ "	$= 2.56 \times 10^{-3}$ "	1.3×10^{-3}	3.6×10^{-3}

[2] Langley, (2), pp. 12, 13, 15.

[3] Langley, (2), p. 20.

[4] Henri and des Bancels, (7), p. 845.

Langley gives another series of figures (2)[1] by which he intends to express "the proportionate results for seven points in the normal spectrum, whose wave lengths correspond approximately with those of the ordinary color divisions, where unity is the amount of energy (about $\frac{1}{1000}$ erg) required to make us see light in the crimson of the spectrum near A." According to this definition, this scale corresponds to the *minimum visibile*.

Wave lengths	0.40	0.47	0.53	0.58	0.60	0.65	0.75
Luminosity (visual effect)	1.600	62.000	100.000	28.000	14.000	1.200	1

Expressed in negative powers of 10, in order to facilitate comparison, we have (unity being 10^{-3} ergs, no indication being given concerning the time):

Wave lengths	0.40	0.53	0.65	0.75
Energy	6.2×10^{-7}	1×10^{-8}	8.3×10^{-7}	1×10^{-6}

The first figure (for 0.40μ) agrees well with that given by Langley (2)[3] in his other tables. The second one (0.53μ) does not agree at all, and the slight difference in wave length cannot be regarded as the cause of the discrepancy. The third one agrees within 25 per cent and the last one also, approximately. We see no explanation for this discrepancy, which cannot be due to a misprint.

Therefore, it was desirable to settle the question, since Langley's data are so misleading that good authors have made errors simply in quoting them. Quite recently, Joly (8) published a very interesting article on a quantum theory of vision, and although he does not share Henri's opinion on the subject, quotes one of his figures, 5×10^{-12} ergs for the threshold of sensitivity for white light. Now, we have tried in vain to find such a figure in two of the papers of Henri and des Bancels, as the indications of the source are missing. As far as we know, they did not make any measurements themselves, but simply quoted those of Grijns and Noyons. They quote the figures given by Grijns and Noyons, 4.4×10^{-11} ergs. Even if we admit that only 10 per cent of the energy is radiated under the form of light (9), we obtain 3.96×10^{-12}, and not 5×10^{-12}. It is regrettable that Professor Joly did not give the bibliographic reference.

Method.

An integration method was used. In other words, a curve representing the intensities of the dispersed beam after its passage through the prism was plotted in function of the wave lengths on coordinate paper. It is clear that the area delimited by this curve and certain

limits, arbitrarily chosen, that is to say, the integral of the curve between these limits, will express the total energy radiated. As the source yields in the same time invisible and visible rays, and as the methods used for measuring the radiation give us figures corresponding to the total radiation, R (visible + invisible), a segment extending between the limits of the visible spectrum must also be integrated. This latter integration gives the quantity of energy spread in the visible part of the spectrum; let it be L. Then the ratio of these two areas will be the luminous efficiency of the source, and will be expressed by $\frac{L}{R} = E$. The percentage of the visible to the invisible is now known. Let us call I the intensities in function of the wave lengths, λ_1 the lower limit of integration, λ_2 the upper limit of integration for the visible, then:

$$E = \frac{L}{R} = \frac{\int_{\lambda_1}^{\lambda_2} I d\lambda}{\int_{o}^{\infty} I d\lambda}$$

The quantity of energy spread by the slit over the visible spectrum being thus known, a suitable screening of each monochromatic light decreases its intensity until the threshold of sensitivity is reached. Knowing exactly the amount of energy absorbed by the screens, the amount which is allowed to pass may be calculated easily: it is the minimum energy necessary to produce visual sensation.

Technique.

Limits of Integration.—Limits of Total Radiation.—Lower limit: For most light sources, the energy in the ultra-violet is so small that the lower limit, 0.4 μ, may be taken as zero without any appreciable error. Gage (10) takes it as the limit in his study of the electric arc, which is one of the richest sources in ultra-violet. The Nernst lamp, on the contrary, yields very little ultra-violet radiation, and it was assumed that this limit could safely be taken. Upper limit: The plotting of energy distribution curves showed that above 7μ in the infra-red, the amount of energy radiated by the Nernst

lamp was very small, as compared to that radiated beyond. From 7 μ to 10 μ, it amounts to less than 1 per cent of the total. As the other errors involved by the method are of a greater order of magnitude, it was adopted as the upper limit, for the total radiation.

Limit between the Red and the Infra-Red.—(Upper limit of integration of the visible spectrum). Langley, although he does not specify it, seems to have chosen 0.75 μ as the upper limit. Many workers have chosen 0.8 μ (as the eye is sensitive to the radiations up to 0.8 μ). Some have preferred 0.76 μ, others 0.7 μ. The reason for the importance of this determination is that energy increases very much between 0.7 μ and 0.8 μ, whereas the impression on the eye is very slightly changed. In other words, the shifting of the limit from 0.8 μ to 0.7 μ will change considerably the amount of energy spent in the visible spectrum, whereas the effect on the eye will hardly be noticeable, since it only brings in very faint, deep red rays which, if absent, do not modify one's impression appreciably. On the other hand, if it is sought to determine the minimum of energy necessary to make the red rays between 0.7 μ and 0.8 μ impress the retina, one has to shift the limit as high as 0.8 μ. And in this case, all the values given for the energy of radiations below 0.7 μ will be altered (by more than 27 per cent). Therefore, in this paper, the two figures are given, so that one may compare the results.

The study of luminosity curves shows that, by removing the part of the spectrum extending beyond 0.7 μ, the total luminosity is only decreased by 0.4 per cent. As König and Brodhun (11) have shown that the human eye was just able to detect a change in luminosity when it amounted to 1.6 per cent, we feel that this limit is advisable.

Measurement of Total Radiation.

The first step was to measure the value in absolute units of the total radiation of the Nernst lamp, with which it was intended to experiment; for it was difficult, owing to the discrepancies found in the figures given by different authors, Lux (12), Hartman (13), Ingersoll (14), etc., to rely upon data found in literature.

The source was an ordinary Nernst lamp, (110 volts, 1.3 amperes). In order to prevent any fluctuations due to cooling by air currents, the glower was enclosed in a brass chamber, with just one rectangular

slit (20 + 3 mm.), in front of the glower. The reflection from the heater and porcelain support was suppressed by fixing the glower by means of its platinum wires at the end of two leads. The inside of the chamber was blackened with soot. A voltmeter was placed across the terminals, and an ammeter in series, so as to know exactly the input in watts. Under normal conditions, it was found to be 87.5 volts × 1.05 amperes = 91.875 watts. These 91.875 watts are not all transmitted by radiation, but part of them are taken away by conduction and convection by the air. Lux and others give the ratio $\frac{\text{input}}{\text{radiation.}}$. But as these figures may correspond to different types of lamps, it was found safer to measure it directly. Besides, this would allow us, by a simple calculation, to check our radiation data against those published previously on the Nernst lamp.

It was first attempted to use a specially made mercury thermometer, with a known weight of mercury in a known weight of glass, blackened on the bulb of which the rays emerging from a 0.1 sq. cm. slit were concentrated by means of a fluorite lens of short focus. This process showed a lack of sensitivity and it was necessary to check it by means of an electric method. Although less difficult to handle than a bolometric device, the following apparatus required a great deal of care and time. A thermopile was made of copper and constantan wires, with ten elements, disposed linearly; the cold ends were simply bent out. On the top of the welded ends, carefully planed and ground, a thin piece of tin-foil exactly 1 mm. wide and 1 cm. long was applied and fixed with a very thin layer of shellac. Then the tin-foil was cut carefully between the welded ends, leaving a little square table of very nearly 1 sq. mm. on each thermocouple. These were blackened with soot, and the whole thermopile fixed in a thermostat. The rays were allowed to fall on the pile through an adjustable slit, and the distance between the source and the couples made equal to 1 meter. The method consisted in compensating the heat generated by the incoming radiation, by the current sent in a strip of constantan placed near the cold ends of the thermocouples, in a tiny calorimeter, 2 cc. in capacity, filled with oil, and well isolated. The following formula was used:

$$E = Ki^2 \frac{\text{cal. gr.}}{\text{0.1 sq. cm. sec.}}$$

K being a constant (function of the resistance of the constantan strip) of the instrument, calculated and experimentally checked, equal to 0.21, we measured a current of 0.0028 amperes; this gives:

$$0.21 \times (0.0028)^2 \frac{\text{cal. gr.}}{\text{sec. } 0.1 \text{ sq. cm.}} = 0.00000165$$

as 0.2388 cal. gr. = 1 watt sec., it corresponds to 0.000694 watts by sq. cm.

Correction for Equatorial Radiation.—This corresponds to the homogeneous radiation of a punctual source of energy of 87 watts; that is, it would require 87 watts from a punctual source to radiate spherically in all directions an amount of energy of that magnitude. We have measured this amount equatorially, that is, normally to a line normal to the glower itself, and, of course, in the best conditions of radiation. But as the beam of light assumes a greater deviation from the equatorial plane, in the case of an incandescent rod, in other words, as the square centimeter exposed to the rays stands higher in latitude on the sphere, the amount of energy radiated is decreased, since the rays are no longer emitted perpendicularly by the rod. Around the two poles, there is even a region where there is no radiation at all. The result is that, whereas the source acts as radiating 87 watts equatorially, it radiates much less as soon as we reach higher latitudes, and becomes zero at the poles, and the mean value of the radiation is much less than 87 watts. It is known that by multiplying the energy radiated equatorially by $\frac{\pi}{4}$, the real value of the radiating energy from the source is known. In this case, $87 \times \frac{\pi}{4} = 68.3$ watts.

Hence, out of the 92 watts sent into the filament, only 68.3 are radiated, and 23.7 are lost by convection and conduction. The ratio $\frac{92}{68.3} = 1.35$ is in excellent agreement with the figure given by Lux: 1.34.

This figure may be checked in another way: the input in the filament being 91.8 watts, roughly 92 watts, it will radiate equatorially $92 \times \frac{4}{\pi} = 118$ watts,

approximately. The quantity radiated actually as measured equatorially, being 87 watts, the ratio $\frac{118}{87} = 1.35$ gives the amount of energy lost.

Calculation of the Luminous Efficiency, and Corrections.—The next step was to determine the ratios

$$\frac{\int_{0.4}^{0.7} I d\lambda}{\int_{0.4}^{7} I d\lambda} \text{ and } \frac{\int_{0.4}^{0.8} I d\lambda}{\int_{0.4}^{7} I d\lambda} = \frac{L}{R}$$

This ratio has been calculated by many authors, Lux, Nichols and Coblentz (15), Ingersoll, Ångström (16), Stewart and Hoxie (17), etc. Their methods were different and their results do not always check perfectly, (some varying by more than 50 per cent, for example, those of Lux and Ångström.) Some of the workers used methods based upon the absorption of one part of the spectrum by water cells in which different substances were dissolved, (copper sulphate, iodine). It has been shown by Nichols and Coblentz that none of these methods based on absorption were reliable. Ingersoll studied the Nernst lamp and published figures of observed luminous efficiency, which vary greatly according to different lamps, and besides, correspond to burners whose consumption was not that of our lamp, (89 watts). Therefore, the energy distribution curve of our burner was plotted by means of a Hilger Infra-Red Spectrometer.[5]

Correction for the Non-Normal Spectrum.—The spectrum was corrected for the lack of homogeneity of the dispersed beam. Indeed, the refracted rays are contracted in certain parts of the spectrum, and expanded in others, so that, for instance, the same slit opening (*e.g.*, 0.25 mm.) covers 0.015 μ on the spectrum at a mean wave length 0.68 μ (from 0.6725 μ to 0.6875 μ), and as much as 0.266 μ, more than 17 times as much, at the mean wave length 2.66 μ (from 2.53 μ to 2.79 μ). This correction was introduced by the consideration of the geometry of the screw motion, (pitch of screw in relation to

[5] Instrument No. 281, Rock salt prism, Angle 59°. 57′. 30″.

rotation of the prism table), and the use of the dispersion formula, given by Paschen (18):

$$n^2 = a^2 + \frac{M_1}{\lambda^2 - \lambda_1^2} + \frac{M_2}{\lambda^2 - \lambda_2^2} - K\lambda^2 - h\lambda^4$$

Then, the range of the spectrum embraced by a given slit was checked by moving a spectrum line across the slit, and reading the result on the drum. The right edge of the *D* lines (sodium), for instance, was brought in contact with the right edge of the slit (0.25 mm. opening), and the reading made. Then it was moved toward the left until the whole *D* line just disappeared, and another reading made. The result was 0.008 μ. This was done for the lines of copper (0.4955 μ, 0.5292 μ), mercury (0.5461 μ), sodium (0.5893 μ), and cadmium (0.6439 μ). For the infra-red, the data are published by Hilger (19).

It was decided to take the area covered at 2.66 μ by a slit 0.025 mm. wide as unit, (0.026 μ) and to fix the slit in such a way, for every wave length, as to cover the same range. A high sensitivity Leeds and Northrup galvanometer was used in connection with the thermopile, (galvanometer resistance = 12 ohms).

Corrections of the Absorptions Due to the Spectrometer.—But, before integrating the plotted curve, another very important correction had to be introduced regarding the absorption by the golden mirrors, because the energy distribution curve does not correspond to the total amount received by the collimator slit, and because the absorption is much greater for short than for long wave lengths. Fig. 1 shows how the absorption varies for different wave lengths.

If R is the coefficient of reflection, and 3 the number of mirrors, the amount of energy reflected is expressed by

$$I = I_0 R^3$$

It is easily seen that for 0.5 μ, for example, only $\frac{1}{9.6}$ of the incident light is transmitted, and much less still for 0.4 μ.

The absorption by the prism amounts to very little. Theoretically, from the formula, $J = J_0 K^c$, where c is the length of the path of light in the rock salt and K a constant equal to 1 between 0 and 9 μ, it is equal to zero. We found that, practically, for the visible,

FIG. 1. Reflection of monochromatic light by gold.

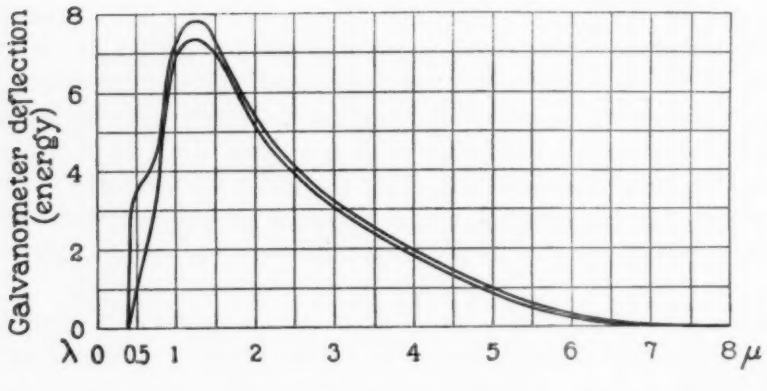

FIG. 2. Energy distribution curves for Nernst lamp, corrected and uncorrected for mirror absorption. Outside curve corrected; inside curve uncorrected.

it really amounts to 1.5 per cent approximately, by concentrating the beam of light before and after passing through the prism.

Finally, the curves were integrated graphically. Fig. 2 shows the different aspects of the curves before and after corrections due to absorption. (These curves are not to scale, in order to emphasize the difference.) The results were:

$$R = \int_{0.4}^{7} = 110.3, \qquad L_{0.8} = \int_{0.4}^{0.8} = 4.8, \qquad L_{0.7} = \int_{0.4}^{0.7} = 3.6$$

The ratios are:

$$\text{Upper limit} = 0.8, \qquad \frac{L}{R} = 0.0435$$

$$\text{Upper limit} = 0.7, \qquad \frac{L}{R} = 0.0316$$

These results are in good agreement with those of Ingersoll, who, with the upper limit 0.76, found figures between 0.036 and 0.046, and in contradiction with those of Drude (20), who gives 12 per cent as luminous efficiency of Nernst lamps, (instead of 4.35 per cent). Lux gives 5.96 per cent, but takes no account of the fact that the screening method he used allowed most of the radiation up to 1.2 μ to pass. Coblentz and Nichols found 0.033 for the efficiency of acetylene flame.

Hence, as we have established that our source radiated 87 watts equatorially, the quantity radiated as luminous waves is equal to 4.35 per cent of 87 (limit 0.8 μ) = 3.8 watts, and 3.16 per cent of 87 (limit 0.7 μ) = 2.75 watts. Let us take the quantity corresponding to the limit 0.7 μ for example. These 2.75 watts are radiated at the distance of 103 cm. from the source, (distance of the thermopile) by square centimeter. The energy then becomes 0.0000206 watts. The area of the slit being 0.1 sq. cm., it only receives 0.00000206 watt seconds, or 20.6×10^{-7} watts = 20.6 ergs. This quantity of energy is spread over the range delimited by the upper and lower limits of integration (0.4 μ to 0.7 μ) and the energy distribution curve corrected for the absorption by mirrors, and by the prism, that is, over a surface of 83 sq. cm., (obtained by graphical integration of the curve, Fig. 3). This corresponds, in the scale chosen, to 0.248 ergs by unit of surface. Similarly, between the limits 0.4 μ

and 0.8 μ, we find 0.343 ergs by unit of surface. In order to determine the efficient amount of energy which will affect the eye, it will easily be seen that this quantity will only have to be multiplied by the segment delimitated on the same chart, by the two ordinates corresponding to the range covered by the slit on the spectrum and the energy distribution curve uncorrected for mirror absorption.

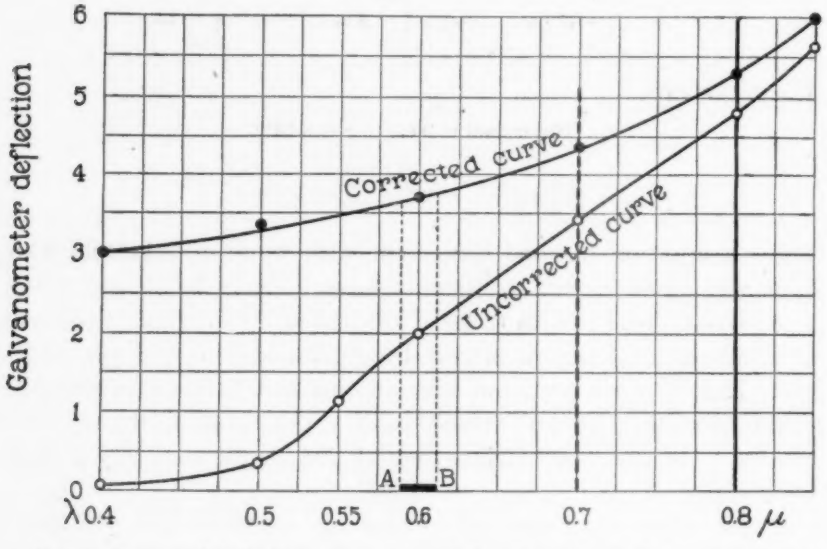

FIG. 3. Energy distribution curve, corrected and uncorrected, in a larger scale in the visible part of the spectrum. *A B* represents the area covered by a slit 0.25 mm. wide.

Test of the Eyes.

In order to reduce the intensity of light by a known quantity, a set of absorbing screens was prepared carefully. It was sought to look directly into the beam of light instead of using reflected light, in order to avoid the errors arising from the reflection of very faint radiations. By getting screens which could decrease by the same known amount, for example, 90 per cent, the intensity of the incident light, the simple formula

$$I = I_0 K^{-n}$$

in which I = emergent light, I_0 = incident light, $K = \frac{I_0}{I}$ and n = the number of screens interposed, leads to this:

$$\text{Log } I_n = \text{Log } I - n \text{ Log } K$$

and as $K = 10$

$$\text{Log } I_n = \text{Log } I_0 - n$$

whence

$$I_n = I_0 \, 10^{-n}$$

The number of screens interposed will itself give the order of magnitude of the out-coming energy: 2 screens will mean that the energy is decreased by 100; 4 screens, by 10,000, etc.

It was found that especially prepared white paper fulfilled the requirements better than any other screen. Sheets of the same paper were chosen, (mean thickness 0.09 mm.), and placed exactly in front of the thermopile, then the throw of the galvanometer was observed; the paper was removed and another reading made at three different wave lengths. A great number were tested, and as we were unable to get ten sheets exactly similar, the thicker were placed on a plane surface and evenly rubbed down with very thin sand, then glossed again with a piece of round glass. They were frequently tested during this process, and finally the following results were considered as satisfactory. (The figures express the ratio $\frac{I}{I_0}$).

TABLE II.

Sheets.	0.55μ
No.	
1	0.100
2	0.103
3	0.103
4	0.095
5	0.0995
6	0.105
7	0.100
8	0.0965
Mean value.....	0.1005

This particular paper was less transparent for the extreme red than for the green, a fact which had to be taken into account.

Measurements.—Eighteen persons were examined; two series of experiments were performed: one after 8 minutes in the dark, and one after 25 minutes. Only five persons were examined for all wave lengths. The others were merely tested for the radiation 0.55. The measurements were carried on in the following way: When nine sheets of paper were placed exactly against the slit, generally no light could be seen. Then, one after the other, the sheets were removed, according to the intensity of light, and usually, owing to the relatively large area (0.1 sq. cm.), there was no difficulty whatever in determining the order of magnitude of the *minimum visibile.* Namely, one sheet added gave a black impression, and this sheet removed left a visible, although very faintly colored, image of the slit. We sought, as Langley did, to determine the *minimum visibile,* defining this to be, not the smallest light whose existence it is possible to suspect, or even to be reasonably certain of, but a light which is observed to vanish and reappear when silently occulted and restored by an assistant without the observer's knowledge (Fig. 4).

On top of the last sheet of paper, another slit was placed, across the first one. Its jaws were cut in such a way that a square opening was left between them, (see Fig. 5); thus a square or rectangular figure was delimited by four moving lines. At first, the slit was adjusted so as to cut a little window of 1 sq. mm. on the luminous spot. If the window could not be seen, the jaws of the slit were moved micrometrically until the spot became visible. The maximum opening corresponded to a vertical motion of 5 mm.

It was found that most women generally require more time than men to reach the same degree of sensitivity. Most of the men tested became adapted in 5 minutes (viz., could see a light corresponding to an energy of the order of magnitude 10^{-9}) while it required 15 to 20 minutes for women to see the same thing. Moreover, some of them could only see the spot spasmodically appearing and disappearing, while men had a continuous impression. Only an increase in the intensity of about 50 to 100 per cent was able to give them the same visual impression. As will be seen, the differences vary between 0 and 25 per cent among men for a given wave length;

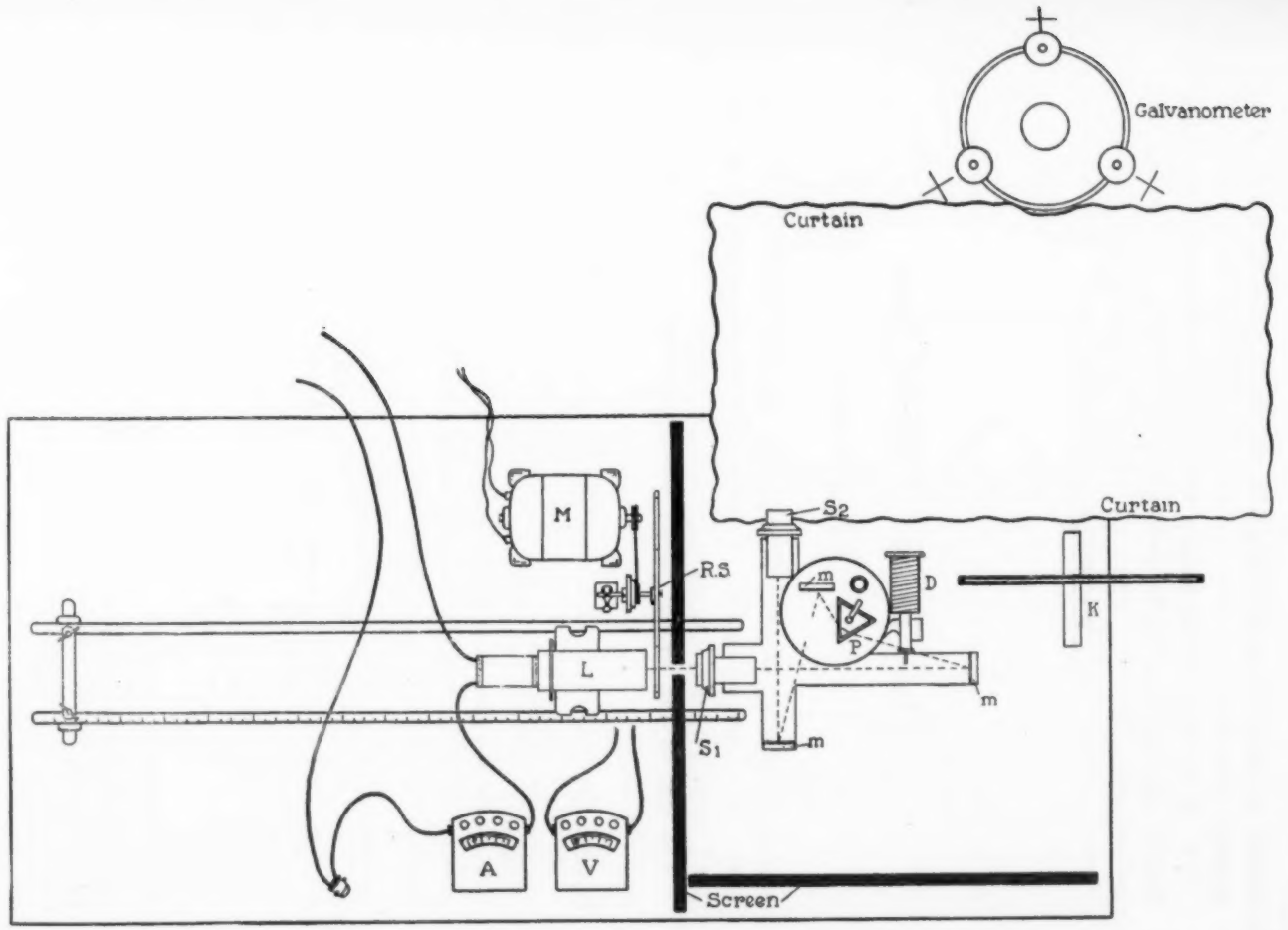

FIG. 4. Apparatus set for the test of the eyes. The observer was looking through slit S_2. *L*, Nernst lamp; *M*, motor; *R. S.* revolving shutter; *A*, ammeter; *V*, voltmeter; S_1, S_2, slits; *P*, rock salt prism; *D*, calibrated drum; *m*, *m*, mirrors; *K*, lamp and scale.

among women, between 0 and 100 per cent One man showed a marked difference.

The figures express the energy necessary for continuous impression but by making the same assumption as Langley concerning the

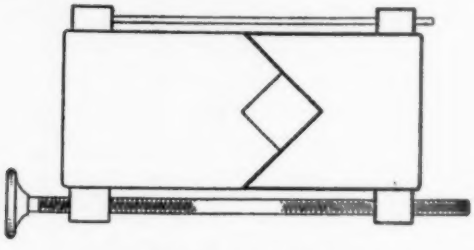

FIG. 5. Adjustable window.

minimum amount of time necessary to perceive distinctly a very faint light, (about $\frac{1}{2}$ second), these figures may be expressed in ergs per $\frac{1}{2}$ second, by dividing them by 2.

The size of the retinal image was approximately 0.01 sq. mm. ± 0.002.

TABLE III.

Men.

Observer.	Age.		0.4 to 0.8 μ	0.4 to 0.7 μ
	yrs.		$\frac{ergs}{sec.}$	$\frac{ergs}{sec.}$
A	29	Normal sight.	7.1×10^{-9}	4.55×10^{-9}
B	36	" "	8.2×10^{-9}	5.9×10^{-9}
C	45	Short sighted.	7.1×10^{-9}	4.55×10^{-9}
D	36	" "	8.3×10^{-9}	5.9×10^{-9}
E	28	Normal sight.	8.3×10^{-9}	5.95×10^{-9}
F	23	" "	8.1×10^{-9}	5.95×10^{-9}
G	19	" "	8.6×10^{-9}	6.1×10^{-9}
H	30	" "	7.4×10^{-9}	5.25×10^{-9}
I*	27	" "	1.0×10^{-8}	8.6×10^{-9}
Mean values.....			8.1×10^{-9}	5.85×10^{-9}

* Observer "I" was included in the mean, although he seemed to be quite out of the normal.

First Series of Experiments.—The observers were protected by a curtain from all light, and waited until their eyes had become quite sensitive before making the experiments. 8 minutes in absolute darkness seemed to be sufficient for men. These first figures will show the difference between the rapidity of adaptation of men and women. Wave length 0.55 μ (Tables III and IV).

TABLE IV.

Women.

Observer.	Age.		0.4 to 0.8 μ
	yrs.		$\frac{ergs}{sec.}$
J	30	Normal sight.	8×10^{-9}
K	30	Short sighted.	2×10^{-8}
L	25	" "	9×10^{-9}
M	22	Normal sight.	1×10^{-8}
N	24	" "	2×10^{-7}
O	23	" "	3×10^{-8}
P	19	" "	2×10^{-8}
Q	42	" "	4×10^{-8}
R	40	" "	5×10^{-8}

Obviously, for women the differences are so great that a mean value would have no significance at all.

It was found that it took over 20 minutes for observers J, M, and O to reach the same sensitivity as men, viz., less than 7×10^{-9}. As Langley does not give any precision concerning the time of adaptation, we may compare his figures to the mean value found for men: Langley (0.55 μ), $5.55 \times 10^{-9} \frac{\text{ergs}}{\text{sec.}}$. We found slightly larger figures:

$$\int_{0.4}^{0.7} : 5.85 \times 10^{-9} \frac{\text{ergs}}{\text{sec.}}$$

$$\int_{0.4}^{0.8} : 8.1 \times 10^{-9} \frac{\text{ergs}}{\text{sec.}}$$

But after more than 20 minutes in the dark, the eye becomes more sensitive still, and we obtained the following figures (Table V). These figures are smaller than those given by Langley, but as he did not state the length of time which the eyes of his experimenters

were kept in the dark, and as we have seen that the sensitivity is increased over 100 per cent by a stay of 25 minutes instead of 8 or 10, they cannot well be compared. Generally, at least, they are of the same order of magnitude for the wave length 0.55 μ. A stay of 1 hour in absolute darkness did not seem to increase the sensitivity beyond these figures.

It must be pointed out that the figures corresponding to the wave length 0.4 μ are doubtful, as the spectrometer which was used was not fit for the measurements in that part of the spectrum, owing to the gilded mirrors. They are only given as approximations. It must also be borne in mind that these quantities of energy do not

TABLE V.

25 Minutes in the Dark. $\int_{0.4}^{0.8}$

Observers.	0.4	0.5	0.55	0.65	0.68	0.72
(*Women*)						
J	2.5×10^{-6}	1.6×10^{-8}	3×10^{-9}	2×10^{-7}	2×10^{-6}	3×10^{-5}
O	5×10^{-6}	2.3×10^{-8}	4.5×10^{-9}	3.5×10^{-7}	5×10^{-6}	4×10^{-5}
(*Men*)						
B	5×10^{-7}	1.4×10^{-8}	2×10^{-9}	1.5×10^{-7}	1.9×10^{-6}	2×10^{-5}
D	5×10^{-7}	1.3×10^{-8}	2×10^{-9}	1.5×10^{-7}	2×10^{-6}	2×10^{-5}
F	8×10^{-7}	1.5×10^{-8}	3×10^{-9}	1.7×10^{-7}	2×10^{-6}	2.5×10^{-5}
Mean values...	3.85×10^{-7}	1.6×10^{-8}	3×10^{-9}	2.2×10^{-7}	2.7×10^{-6}	2.5×10^{-5}

correspond exactly to one pure radiation of wave length, 0.55 μ for instance, but to the beam comprised between 0.537 μ and 0.563 μ, the slit covering a range of 0.026 μ.

White Light.—The same technique was applied to the *minimum visibile* for white light (Nernst lamp), and gave 3.8×10^{-11} ergs, for continuous impression by total radiation. This figure agrees well with that of Grijns and Noyons for the Hefner lamp, 4.4×10^{-11}. It is better to compare figures related to total radiation, because these figures do not involve the more or less arbitrary choice of limits of integration and the knowledge of the ratio $\frac{L}{R}$ for the considered source.

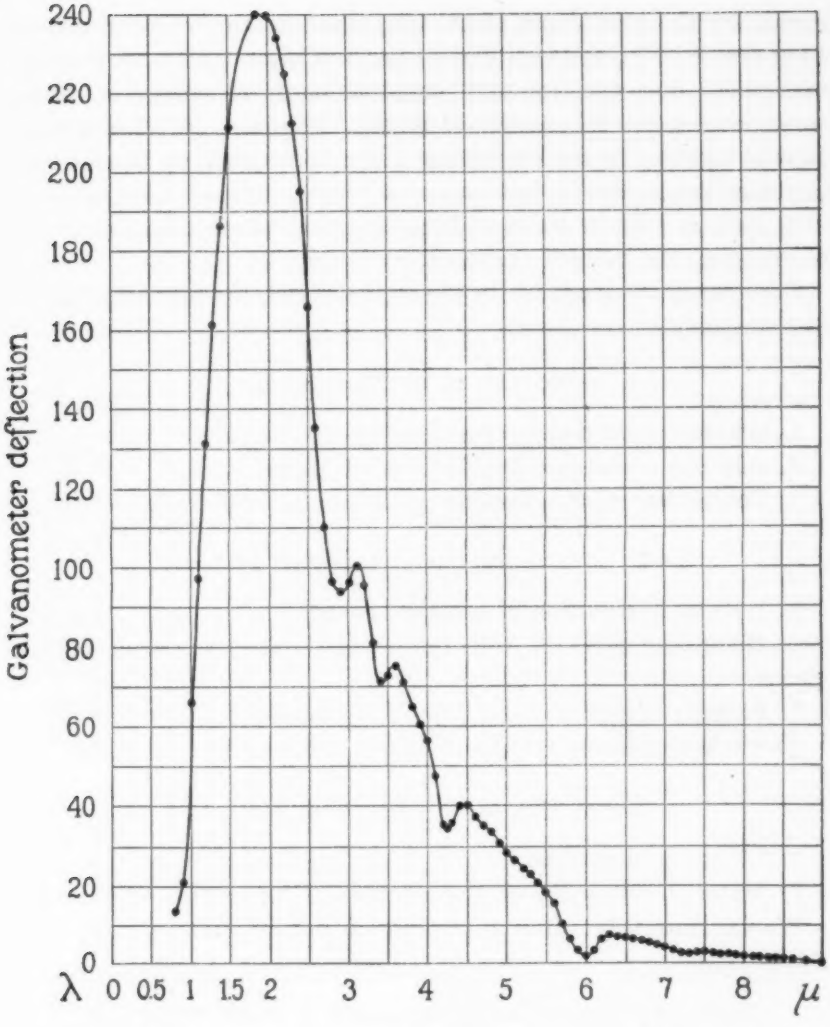

FIG. 6. Energy distribution curve of the Nernst lamp used (uncorrected), 91.8 watts: slits: 0.025 mm.

Criticisms of the Results.—As has been pointed out before by Langley, the errors involved in the determination of the threshold of sensitivity (*minimum visibile*) may be perhaps 100 per cent, or even more. For this reason, the absorption by the various eye

media, for the total depth of the eye, which amounts only to about 1 per cent for 0.7 μ and less than 0.1 per cent below 0.65 μ, are entirely negligible. The eye, for such small amounts of energy as those corresponding to the *minimum visibile*, does not perceive a continuous increase in the brightness of the spot, when its intensity is increased progressively, but seems to react by steps. High authorities, such as Joly, and Henri, disagree entirely as to the explanation of vision on the basis of the quantum theory.

Accuracy of the Method.—In the method used, the following causes of error could be corrected:

Errors Due to the Spectrometer.

1. Selective reflection by the three gilded mirrors.
2. Selective absorption by the rock salt prism.
3. Contraction of the spectrum, (non-normal spectrum).

Errors Due to the Use of a Nernst Filament.

1. Uneven distribution of spherical energy.
2. Disturbing effect of volt- and ammeter in the circuit of the glower.
3. Radiant output of glower, (losses by conduction).

The following errors are also involved, and were not corrected:

Errors Due to the Integration Method.

1. Determination of limits of integration, (arbitrary).
2. Material errors due to the mechanical integration of surfaces.

We can probably admit that they do not amount to more than 10 per cent.

Errors Due to the Assumed Quantity of Energy Radiated.

1. Errors due to the fact that the image of the glower was not formed on the collimator slit.
2. Errors due to the emission of radiations from other parts of the instrument.
3. Errors due to the measurement of the total radiation by means of a compensating current.

As our figures are in good accord with those of the best authors, within less than 10 per cent, we may assume that this is the upper limit of error. This gives a total of 20 per cent possible error, which is beyond the possibility of detection by the eye in the *minimum visibile*, as stated before. As some of the individual data differ by more than 100 per cent, the data can only be considered as reliable in the conditions of the experiments, within about 120 to 150 per cent. This is about the order of magnitude of the differences between the experimental data given by Langley.

Quantum Theory.—We can roughly express the *minimum visibile* in function of quanta of energy. For the mean radiation 0.55 μ, the period of the atom is 5.76×10^{14} per second. The minimum of energy perceived is approximately equal to 1.9×10^{-12} ergs per second, (taking 3.8×10^{-11} as the value of the minimum for total radiation, and roughly 5 per cent as belonging to the visible spectrum). Hence,

$$\frac{1.9 \times 10^{-12}}{5.75 \times 10^{14}} = 3.3 \times 10^{-27}$$

As Planck's universal constant $h = 6.5 \times 10^{-27}$, the figure found is satisfactory as far as the order of magnitude is concerned, but it would mean that only one-half quantum per second would be sufficient to cause the luminous sensation; as we have dealt with an area of $\frac{1}{100}$ of a square millimeter on the retina, it would indicate that the destruction of one molecule every 2 seconds on such an area would be sufficient to produce an impression of light.

CONCLUSION.

A method was devised for measuring the *minimum visibile* in different parts of the spectrum, as done by Langley in 1888.

The results are generally in good agreement with those given by this author, although not as close on both sides of the wave length 0.55 μ; this may be due partly to the use of a rock salt prism, to the fact that the minimum was determined by looking at a beam of diffused transmitted, instead of diffused reflected light, and also to the fact that Langley experimented with the sun, through the earth's atmosphere, and had to take into account the thickness of the atmos-

phere interposed and the brightness of the sky. Although his experiments were made with great care, the differences from one day to another are important. However, when he expresses the energy in absolute units, he always refers to the same mean amount of energy radiated by the sun on 1 sq. cm. This amount is certainly not constant, if one judges from the differences observed in two measurements of sensitivity of the eye of the same individual at different dates. On the contrary, for a given wave length, our measurements always agreed closely, as our source of radiation was very nearly constant, owing to the absence of a varying amount of water vapor interposed. This may in some way account for the discrepancies observed.

I wish to express my thanks to Dr. Harry Clark of The Rockefeller Institute for the valuable help he was kind enough to give me in solving certain difficulties which were encountered during this work.

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THE RATE OF GROWTH OF THE DOMESTIC FOWL.

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If the weight of a mammal, for example the mouse, is plotted against its age, the resulting curve will be seen to show three fairly distinct waves, oscillations, or growth cycles. These waves or cycles are still more strikingly shown by plotting the velocity of growth against age as is seen in Fig. 1.

Robertson[1,2] found that the growth of each of these cycles can be represented by the equation of an autocatalytic monomolecular reaction. This fact, and the theories developed from the fundamental studies on cell growth by Sachs, Morgan, Driesch, Boveri, and Loeb,[3] which were reviewed in a previous paper[4] on the growth of the dairy cow, led Robertson[1,2] and Ostwald[5] to assume that the limiting factor of growth of each cycle is an autocatalytic monomolecular reaction. Assuming this tricyclic and autocatalytic theory of growth to be true for mammals, it becomes of interest to find out whether the same does not also hold for birds. The object of this paper is to examine data on the growth of the domestic fowl in the light of this theory.

Fig. 2, deduced from Table I, shows the rate of growth of the Rhode Island Red breed of fowl[6] from the time of hatching up to the age of

[1] Robertson, T. B., *J. Biol. Chem.*, 1916, xxiv, 363.

[2] Robertson, T. B., *Arch. Entwcklngsmechn. Organ.* 1907–08, xxv, 581; *Am. J. Physiol.*, 1915, xxxvii, 1, 74; Principles of biochemistry, for students of medicine, agriculture and related sciences, Philadelphia, 1920.

[3] Loeb, J., The dynamics of living matter, New York, 1906, 58–66; *Biochem. Z.*, 1907, ii, 34; *Biol. Centr.*, 1910, xxx, 347.

[4] Brody, S., and Ragsdale, A. C., *J. Gen. Physiol.*, 1920–21, iii, 623.

[5] Ostwald, W., Vorträge and Aufsätze über Entwicklungsmechanik der Organismen, Leipsic, 1908, v.

[6] Card, L. E., and Kirkpatrick, W. F., *Storrs Agric. Exp. Station, Bull. 96*, 1918.

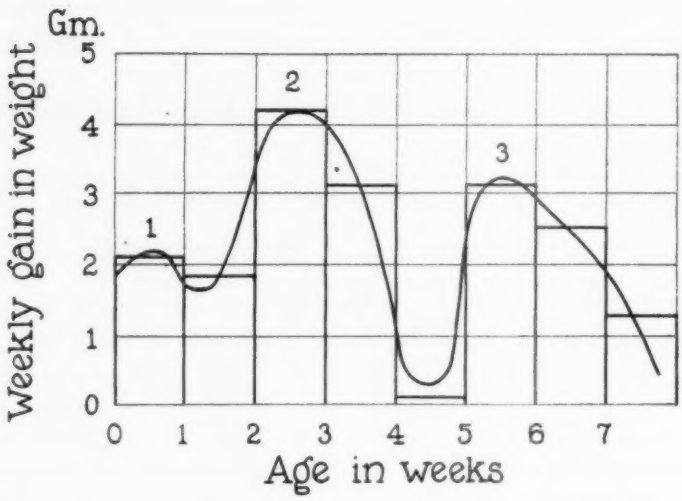

FIG. 1. The rate of growth of the white mouse plotted from data by Robertson.[1] Ordinates represent the weight (x) in grams gained per week; abscissæ represent the age (t) in weeks of the animals.

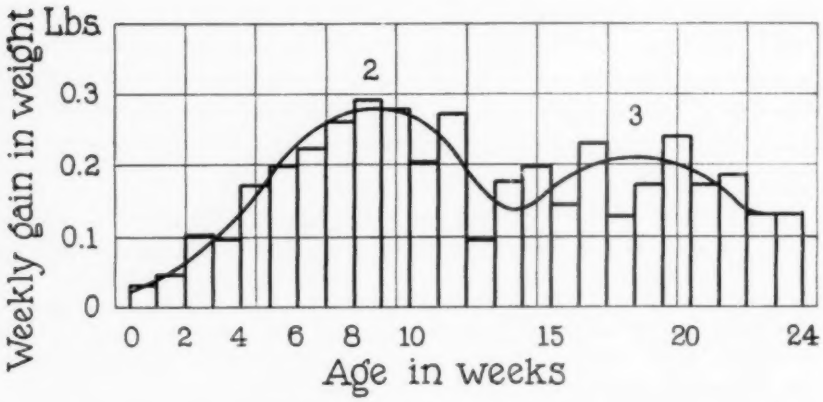

FIG. 2. The rate of growth of the Rhode Island Red fowl plotted from data by Card and Kirkpatrick.[6] Ordinates represent the weight (x) in pounds gained per week; abscissæ represent the age (t) in weeks of the birds from the time of hatching.

laying. This figure clearly shows the rate of growth to increase from the time of hatching up to about 8.5 weeks of age when the rate of

TABLE I.

Growth of the Rhode Island Red birds.			Growth of chick embryo.			
After Card and Kirkpatrick.[8]			After Lamson and Edmond.[9]		After Hasselbalch.[10]	
Age.	Weight.	Number of birds.	Age.	Weight.†	Age.	Weight.
wks.	*lbs.*		*days*	*gms.*	*days*	*gms.*
0 (hatched)	0.082	865			3	0.004
1	0.115	858	4	0.12	4	0.054
2	0.162	798	5	0.28	5	0.155
3	0.264	750	6	0.49	6	0.373
4	0.364	714	7	0.86	7	0.615
5	0.538	696	8	1.35	8	1.200
6	0.737	682	9	1.98	9	2.040
7	0.962	677	10	2.85	10	2.890
8	1.228	613	11	3.95	11	4.366
9	1.525	611	12	5.89	12	5.674
10	1.805	608	13	8.21	13	7.543
11	2.014	601	14	9.84	14	10.005
12	2.290	600	15	12.63	15	12.285
13	2.388	344*	16	16.05	16	15.210
14	2.566	343	17	20.05	17	17.500
15	2.765	343	18	21.36	18	21.545
16	2.907	288*	19	26.87		
17	3.136	288	20	35.50		
18	3.265	288				
19	3.436	253				
20	3.677	252				
21	3.851	251				
22	4.035	250				
23	4.165	250				
24	4.296	250				

* The males were removed at the end of the twelfth and fifteenth weeks. The other reductions in number are due to removal of chicks by crows and necessity of removing chicks due to limitations of range conditions.

† Average of ten embryos.

growth is at a maximum; from this maximum at 8.5 weeks the rate of growth decreases to a minimum at about 14 weeks. This is followed by another cycle with a maximum at about 18 weeks of age ending

somewhere between 24 and 28 weeks[6] of age when growth of non-fatty tissues probably ceases. That each of these postembryonic cycles follows the equation of an autocatalytic monomolecular reaction can be seen from their shape. This can be easily verified by taking t_1, the age of maximum growth at 8.5 weeks, and following the technique of computation described in the preceding paper on the growth of the dairy cow, we obtain the equation

$$\text{Log } \frac{x}{2.76 - x} = 0.182\ (t - 8.5)$$

which gives calculated values closely agreeing with the observed weights.

Age (t).	Observed weights.	Calculated weights (x).
wks.	*lbs.*	*lbs.*
0	0.082	0.078
1	0.115	0.114
2	0.162	0.168
3	0.264	0.251
4	0.364	0.363
5	0.538	0.516
6	0.737	0.717
7	0.962	0.961
8	1.228	1.233
9	1.525	1.521
10	1.805	1.800
11	2.014	2.040
12	2.290	2.240
13	2.388	2.390
14	2.566	2.505

Essentially similar results were obtained with other breeds of fowl such as the White Leghorn,[6] single comb White Leghorn,[7] and White Plymouth Rocks,[8] the several breeds differing only with respect to the several constants. From Fig. 2 it is clear that the equation will also hold somewhat less accurately for the second cycle with a maximum at 18 weeks.

[7] Lamon, H. M., and Lee, A. R., *U. S. Dept. Agric., Bull. 561*, 1917, 37.

[8] Phillips, A. G., *Purdue Univ. Agric. Exp. Station, Bull. 1906*, 1916; *Purdue Univ. Agric. Exp. Station, Bull. 214*, 1918.

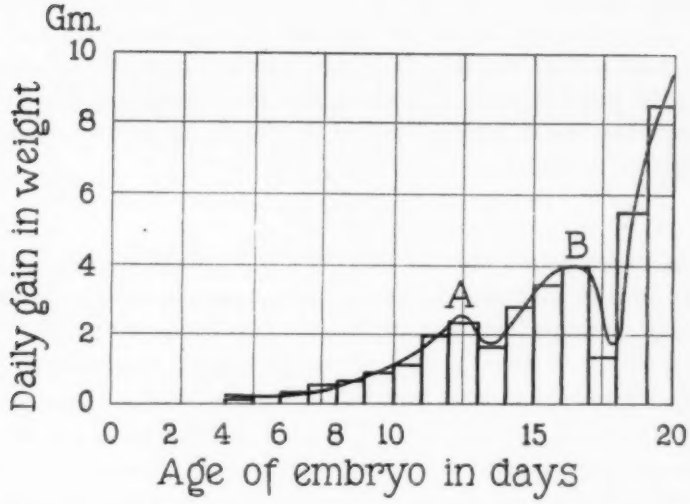

FIG. 3. The rate of growth of the chick within the egg plotted from data by Lamson and Edmond.[9] Ordinates represent the weight (x) in grams gained per day; abscissæ represent the age (t) of the embryo from the beginning of incubation.

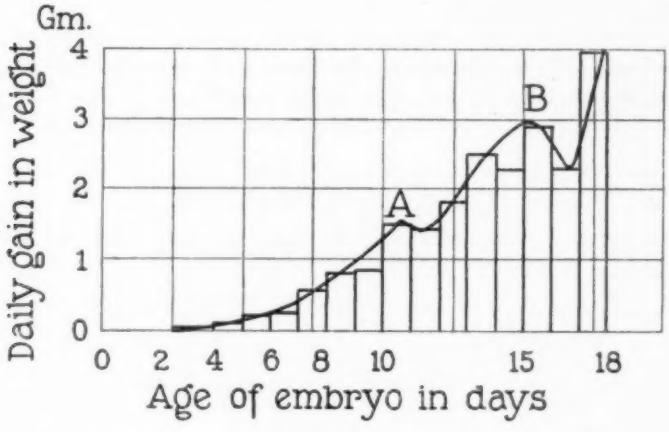

FIG. 4. Same as Fig. 3 plotted after data by Hasselbalch.[10]

The mammal was seen to go through at least three cycles during its growth period; if the tricyclic theory of growth is also true for the bird, then the fowl must go through a complete cycle during its embryonic period of growth. Figs. 3 and 4, curves of the embryonic period of growth, show this to be the fact;[9,10] indeed it seems as if the curve of the embryonic period of growth shows two cycles with maxima at A and B.

SUMMARY.

This paper points out the fact that the growth period of the domestic fowl is analogous to that of the mammal, being composed of three, or perhaps four, cycles; two of these cycles are postembryonic with maxima at about 8 and 18 weeks varying somewhat with the breed and two or at least one, are embryonic with maxima at 11 to 12 and 15 to 16 days of age. Hatching occurs during the first part of the second or third cycle resembling in this respect the guinea pig[11] rather than the mouse.[1] The velocity curves of each of these cycles are similar to and can be represented by the equation of an autocatalytic monomolecular reaction.

The author is indebted to Mr. H. L. Kempster, Department of Poultry Husbandry of this Station for supplying him with some references in this paper.

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THE THERMOLABILITY OF COMPLEMENT, IN RELATION TO THE HYDROGEN ION CONCENTRATION.

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(Received for publication, May 12, 1921.)

That property of fresh blood serum by which it is able to take part in, and complete various immunological reactions is known as complement. The thermolability of this function is well known. In respect to the separated fractions, that contained in the euglobulin fraction of the serum and known as mid-piece, and that contained in the pseudoglobulin and albumin fraction and referred to as end-piece, Ferrata[1] found only the end-piece thermolabile. Subsequent observers have uniformly found both fractions thermolabile. A number of investigators[2, 3, 4] have noted that the mid-piece is more resistant when heated in whole serum than when isolated. Leschly[5] found moreover that the mid-piece, apparently inactivated by brief heating to 56°C. was able to complete hemolysis when added separately to the sensitized cells, the addition of end-piece being made after an interval, so that under these conditions there is not a permanent destruction, but the development of the modification described by Brand.[6]

With regard to the effect of the chemical reaction it has been known since the observations of Ehrlich and Morgenroth[7] and Ehrlich and Sachs[8] that the addition of considerable amounts of acid or alkali

*Van Cott Fellow in Pathology.

[1] Ferrata, A., *Berl. Klin. Woch.*, 1907, xliv, 366.

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[3] Marks, H. K., *Z. Immunitätsforsch. Orig.*, 1911, xi, 18.

[4] Mutermilch *Compt. Rend Soc. de Biol.*, 1911, lxx, 577.

[5] Leschly, W., *Z. Immunitätsforsch. Orig.*, 1916, xxv, 44.

[6] Brand, O., *Berl. Klin. Woch.*, 1907, xliv, 1075.

[7] Ehrlich, R. and Morgenroth, J., *Berl. Klin. Woch.*, 1899, xxxvi, 481.

[8] Ehrlich, R., and Sachs, H., *Berl. Klin. Woch.*, 1902, xxxix, 297.

permanently inactivate complement. The destructive or inhibitory effect of slight degrees of acidity or alkalinity has been noted by a number of later workers. Liefmann and Cohn,[9] and Guggenheimer[10] found that the isolated mid-piece is more affected by the chemical reaction than is the end-piece; both fractions are more affected by acid than by alkali. Michaelis and Skwirsky[11] and Leschly[5] have worked with definite H ion concentrations, but their results do not relate to the present problem. Brooks[12] has recently defined the limits of pH value of the acidity beyond which complement is permanently inactivated.

The salt concentration of the solution has been found to affect complement in a way which appears to be quite different from that in which the two fractions of complement are separated by dilution with distilled water and dialysis, as in the original method of Ferrata.[1] Sachs and Teruuchi[13] found that when fresh serum is diluted 1 to 10 with distilled water a permanent inactivation occurs, and takes place more rapidly at 37°C. than at lower temperatures.

In the present work the degree of complementary activity retained by fresh guinea pig serum after being heated to various temperatures in solution in distilled water or in saline solution, at definite H ion concentrations, has been determined by a modification of the method of Brooks.[14] This consisted in measuring in a colorimeter to provide a standard for comparison the percentage of hemolysis given by the following amounts: 1, 0.8, 0.6, 0.4, 0.3, 0.2, 0.1, and 0.05 cc. of a dilution of each specimen of serum in saline solution. This dilution was first made 1 to 20, and then slightly increased to correspond exactly to the final dilution of the samples which were heated under varying conditions of temperature and reaction. The degrees of hemolysis thus observed were plotted as ordinates against the amounts of complement dilution as abscissæ. From the curve so obtained the relative efficiency or activity of each

[9] Leifmann H., and Cohn, M., *Z. Immunitätsforsch. Orig.*, 1910, vi, 562.
[10] Guggenheimer, H., *Z. Immunitätsforsch. Orig.*, 1911, xi, 393.
[11] Michaelis, L. and Skwirsky, P., *Z. Immunitätsforsch. Orig.*, 1910, vi, 357, 629
[12] Brooks S. C. *J. Gen. Physiol.*, 1919–20, 185.
[13] Sachs, H., and Teruuchi, Y., *Berl. Klin. Woch.*, 1907 xliv, 467.
[14] Brooks, S. C., *J Med. Res.*, 1919–20, xli, 399.

heated sample can be obtained by reading the abscissa at which the percentage of hemolysis given by 1 cc. of the diluted and heated specimen intersects the base-line curve. The various corrections used by Brooks were not made. By the use of this principle as Brooks has shown, one compares amounts which produce like results, and can thereby obtain the actual ratio between the unheated control and the heated sample with respect to the complementary function under consideration. For each complete experiment there was used one stock emulsion of 5 per cent sheep cells sensitized with two units of sensitizer. The total volume in each tube of the titration and of the test was 5 cc. before the addition of acid or alkali.

The H ion concentration was determined by diluting three 2.5 cc. portions of each specimen of serum to 5 cc. with distilled water or saline, according to the medium chosen, adding methyl red and bromthymol blue separately to two of the tubes and determining by comparison with a standard series the H ion concentration reached after the addition of successive drops of N/40 HCl in equal number to each tube. The third tube served as a color screen after each addition of acid.[15] For each specimen of the isolated fractions of complement a similar titration was carried out, using for the end-piece N/40 and for the mid-piece N/160 HCl and NaOH.

The partition of the complement into the fractions was carried out by bringing the fresh serum diluted 1 to 20 with cold distilled water to a pH between 6.2 and 6.4, using for this purpose the necessary amount of HCl as calculated from the titration described above. This narrow range of reaction is that which was found optimal for the precipitation of the euglobulin under these conditions. A titration was found necessary for each specimen of serum because of differences in the amount of acid required to bring the pH to the desired reaction, and the blood of a single animal for the same reason was used for each experiment. The different methods which have been used for the partition of complement obviously depend upon the insolubility of the euglobulin within a narrow range of reaction

[15] Electrometric control of the colorimetric determinations indicate that the limit of error in the latter is pH 0.2. Between pH 5.8 and 6.5 the error is consistent in sign and lies between +0.15 and +0.10, the electrometric pH is more acid. No corrections have been made on the curves given here.

in a salt-poor medium. After bringing the serum dilution to the desired reaction it was left standing 1 hour on ice and the flocculent precipitate brought down by centifugation. The supernatant fluid was decanted, the inner walls of the tube wiped dry with a clean cloth and the sediment representing the mid-piece used at once or after a single washing with distilled water of pH 6.0. The supernatant fluid from the first sedimentation was used as the end-piece dilution. The final preparation of mid-piece was made by carefully emulsifying the sediment in distilled water, or by subsequently bringing it into solution by the addition of NaOH to pH 7.4.

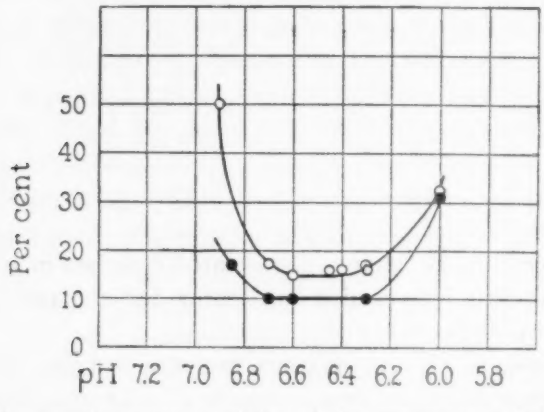

FIG. 1. The complementary activity of the supernatant fluid alone, after precipitation of the euglobulin at different reactions. One experiment is represented by the lower curve and two by the upper curve.

Many workers have had difficulty in separating completely the fractions of complement. The extent to which the completeness of separation depends upon the hydrogen ion concentration at which the separation takes place, is shown by the curve, Fig. 1. This records the complementary activity of the supernatant fluid alone separated at different reactions from the euglobulin sediment.

After adding the amounts of acid or alkali necessary to produce the desired reaction, to a series of tubes containing the different complement dilutions, the tubes were heated in all cases for 10 minutes in a water bath, then cooled by placing in ice water. The fluids in the tubes were all brought to the same reaction (pH 7.3 in the case of whole

serum, pH 6.3 in the case of the fractions) the volumes equalized, and where distilled water had been used as the diluent, isotonicity was restored by the addition of one-tenth volume of 8.5 per cent NaCl solution. The original dilution was 1 to 20 in all cases, and the fractions were used in the same corresponding dilution. Between manipulations the tubes were placed in ice water and protected from sunlight.

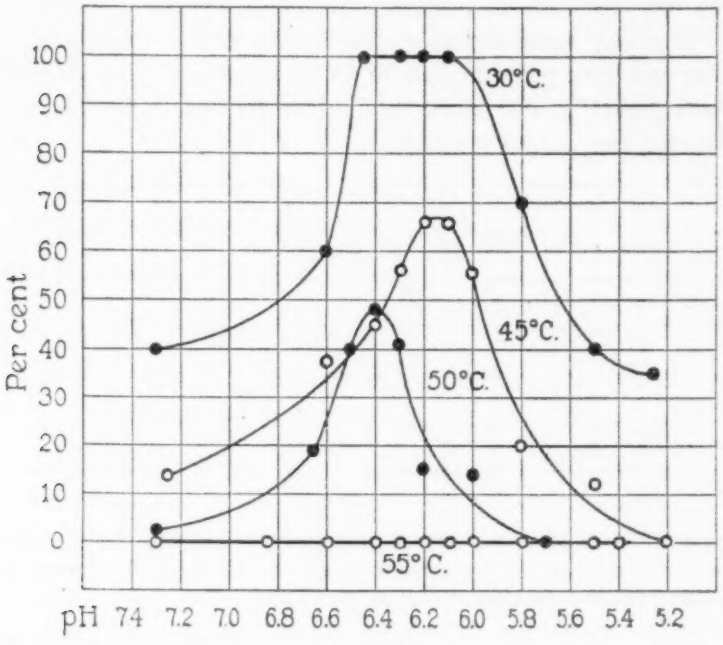

FIG. 2. The complementary activity retained by whole serum diluted in distilled water and heated at different temperatures, at varying reactions.

The complementary activity in percentages of that of the unheated control retained by whole serum diluted 1 to 20 in distilled water and heated at varying reactions at different temperatures, is shown in Fig. 2. The experiments recorded gave the highest values observed in many similar experiments. The value at any given pH relative to the unheated control varies with different specimens of serum, so that each curve is given to represent a single experiment. Although the amount of destruction increases with the temperature it is possible on account of this individual variation in thermolability,

which has been noted by all observers, to make accurate comparison only of the relative degrees of inactivation at the different reactions. In all experiments it was found that the destruction of complement is least at a point between pH 6.1 and 6.4. Within this narrow range from 20 per cent to 50 per cent of the original, activity may be retained when the complement is heated to 50°C. for 10 minutes. On either side of the optimal reaction the drop in activity is rapid. The curves run approximately parallel for all temperatures of heating. At 53°C. a very small degree of hemolytic activity is retained at the optimal reaction; when heated to 55°C. for 10 minutes all complementary power is lost, when referred to the small amount of sensitizer used here, at all reactions.

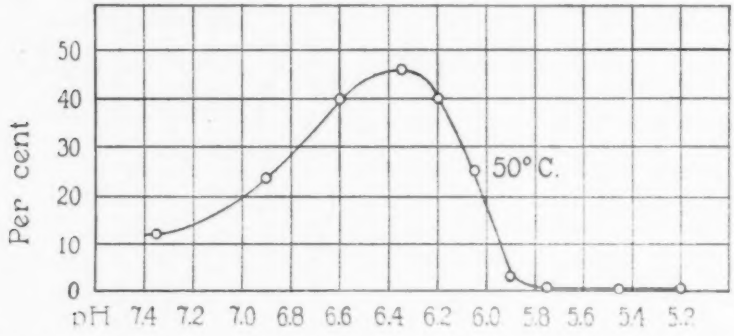

FIG. 3. The complementary activity of the euglobulin fraction in the presence of added end-piece, after heating whole serum to 50°C. in distilled water at the pH values given, and then separating the euglobulin.

If after heating the serum dilution the euglobulin is separated by bringing the reaction in all the tubes to pH 6.3 and removing the sediment by centrifugation, the complementary power of this euglobulin in the presence of the titer amount of end-piece follows the curve given in Fig. 3. It is evident then that the preservation of the complementary power at pH 6.1 to 6.4 depends upon preservation of the mid-piece function at these reactions.

A second maximum in the preservation of the complement was found in numerous experiments at pH 6.9, as shown in the lower curve in Fig. 4, from a typical experiment. The importance of this particular reaction was not appreciated in the early experiments

in which it was not included. It depends apparently upon the greater preservation of the end-piece function at this reaction. This will be referred to later. If the heated complement be added, without separation into its fractions, to sensitized cells together with unheated mid- and end-piece in separate series, the upper curves of Fig. 4 result. The two maxima observed at pH 6.9 and at pH 6.1 to 6.4 appear to be an expression of the quantitative relations between the two fractions, according to which the degree of hemolysis is in-

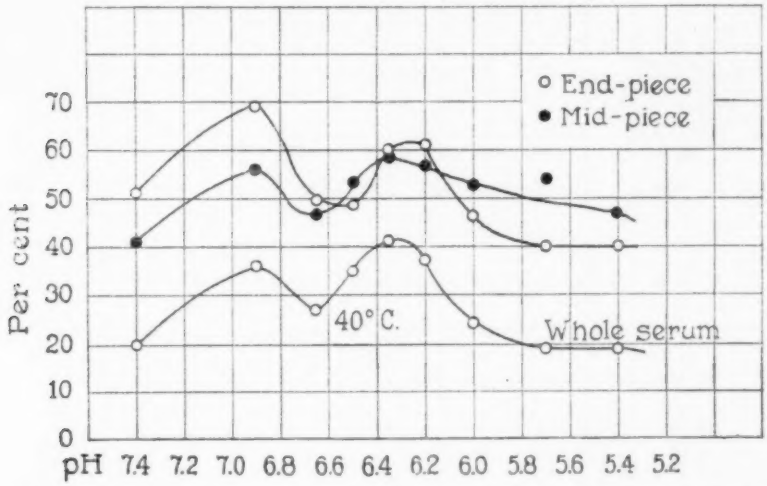

FIG. 4. The complementary activity of whole serum heated to 40°C. in distilled water dilution, by itself and in the presence of added mid- and end-piece. The curve marked end-piece is that given after the addition of the titer amount of mid-piece to each tube; that marked mid-piece is that given after the addition of the titer amount of end-piece to each tube.

creased if the amount of either mid- or end-piece is increased up to the titer value of each.[5]

The activity retained by the isolated end-piece fraction, freed from mid-piece by the precipitation of the euglobulin and heated in dilution in distilled water is shown in Fig. 5. In the experiments recorded here with the isolated fractions the original activity of the complement was completely restored on reuniting the unheated fractions, and the curves that show the effect of heating are essentially the same whether the activity is estimated from the base-line

curve given by the unsplit and unheated control or from that given by varying amounts of one fraction and constant amounts of the other fraction, in each tube of the control titration. It is evident that there is a broad optimal zone for the preservation of the end-piece function, with a reaction of about pH 6.9 as its central and highest point. At this reaction the end-piece is relatively thermostable as compared with whole complement.

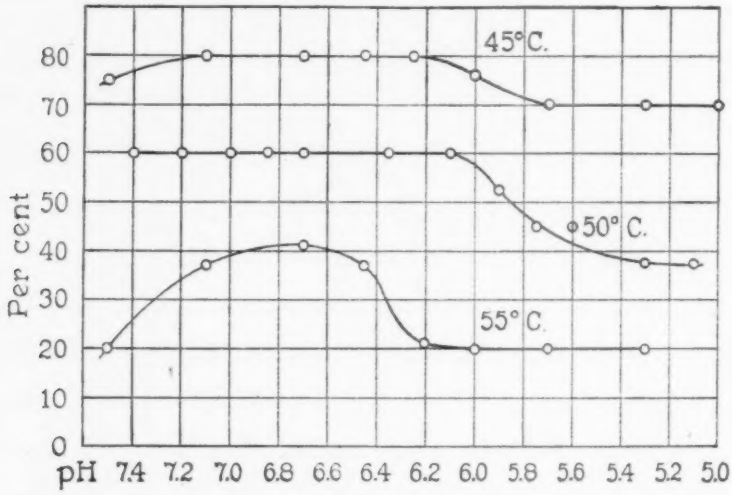

FIG. 5. The complementary activity in the presence of unheated mid-piece of the end-piece fraction heated separately in distilled water dilution at different reactions.

A similar relative independence of the H ion concentration as compared with whole complement is shown by the isolated mid-piece fraction, heated in distilled water. Two experiments at 50°C. are shown in Fig. 6. There is an optimal zone about pH 6.2 with a gradual fall on the alkaline side and a more rapid fall on the acid side of this point.

In the experiments with the isolated fractions the mid-piece whether heated or unheated was added first to the sensitized cells in order to avoid the peculiar modification described by Brand.[6] The curves represent therefore the degree of complete or irreversible inactivation of the fractions.

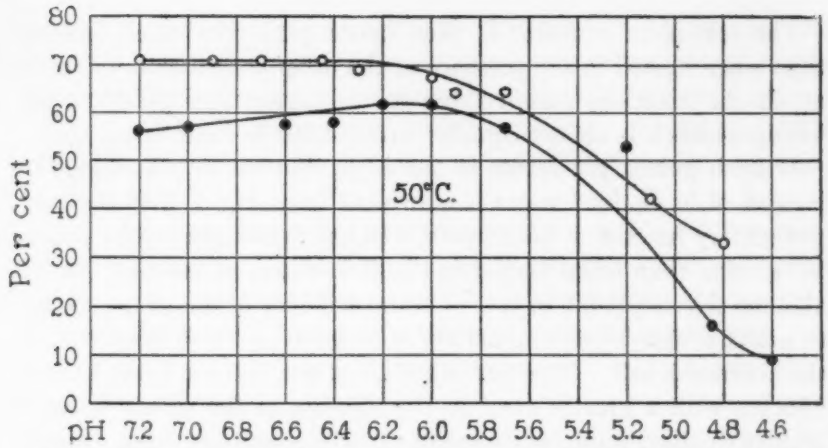

FIG. 6. The complementary activity in the presence of unheated end-piece of the euglobulin (mid-piece) fraction heated separately in distilled water at different reactions.

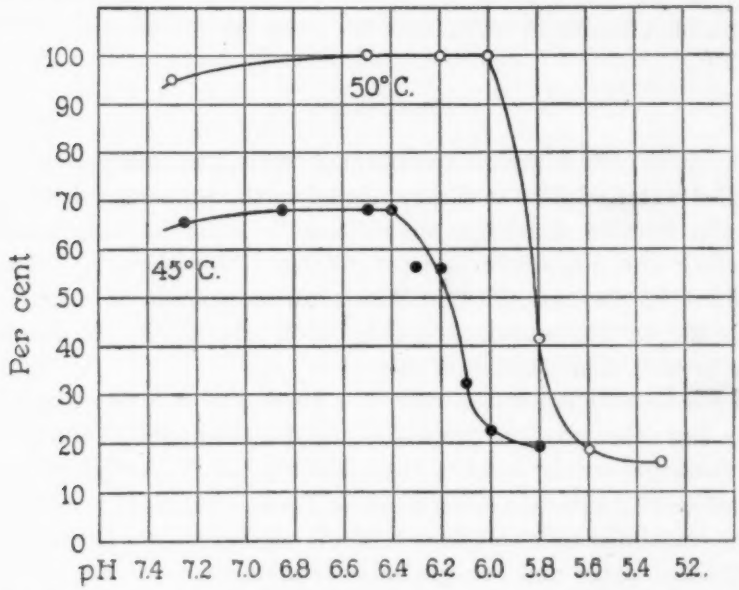

FIG. 7. The complementary activity of whole serum heated at different reactions in isotonic saline solution. The individual variation in thermolability is shown by the different maxima reached in the two experiments recorded here, the lower values being given in this case by the serum which was heated to the lower temperature.

The mid-piece function is thus better preserved when isolated than when heated in the presence of the other constituents of serum at all the reactions except those in the narrow zone pH 6.1 to 6.4, within which it is almost equally thermostable in both cases.

If fresh guinea pig serum is heated in dilution in saline solution instead of in distilled water it is evident from Fig. 7 that the complementary function is better preserved and is less affected by change in reaction than when heated in distilled water, at reactions on the alkaline side of pH 6.1 to 6.4. On the acid side of this point the loss in activity with increasing acidity is as great in the presence as in the absence of salt. The determination of the H ion concentration is affected with a greater error in the dilution of the serum in saline than in distilled water, when colorimetric methods are used. Nevertheless the turning-point observed is essentially the same as in the experiments in distilled water dilution, and corresponds with the point on the curve given by Brooks[12] at which the destruction of complement begins in saline solution when the temperature is kept at 10°C.

DISCUSSION.

The inactivation which complement undergoes when heated in distilled water dilution is closely related to the properties of the euglobulin fraction since this destruction is least at the reaction at which the euglobulin is least soluble. The chemical reaction involved in thermolability in which the euglobulin is necessarily one of the reacting substances is therefore determined by the chemical state in which the euglobulin exists.

It has been found in the case of a number of proteins that solubility and other physical properties are at a minimum at the H ion concentration characteristic of the isoelectric point. At this point the ionization of the protein either as an acid or as a base is at a minimum. If the euglobulin sediment from serum is washed with a large volume of distilled water and brought into solution again by the addition of NaOH to pH 7.4 it becomes least soluble on the addition of HCl between pH 5.1 and 5.7 (depending probably on its purity) and is isoelectric about pH 5.0. The latter determination was made electrometrically; the value corresponds closely with that given by Rona

and Michaelis[16] for serum euglobulin (about pH 5.2). When examined in whole serum however the euglobulin precipitates best at pH 6.3 as noted above and shows no movement in the electric field between pH 6.2 and 6.4. Under these conditions it exists therefore probably not as pure euglobulin but as a compound with some other substance of the serum.

Different compounds of a protein may differ in the position of the isoelectric point. When the reaction of red blood cell suspensions in saccharose solution is adjusted with NaOH and HCl the isoelectric point as determined by cataphoresis and the optimum for agglutination lie near pH 4.7;[17] in the presence of sodium acetate and sodium phthalate this critical point is found near pH 3.8.[18] The compound with a weak acid thus differs from the compound with a strong acid, as pointed out by Professor J. L. R. Morgan;[19] it is possible then that the value pH 6.1 to 6.4 represents the critical point in the ionization of a compound of the euglobulin with a weak base, in which state it exists in the serum or when dissolved or suspended in distilled water without washing. We may conclude then that it is chiefly or entirely the ionized fraction of the euglobulin which takes part in the reaction involved in thermolability.

The existence of a point of inflection in the thermostability of the end-piece at pH 6.9 suggests a similar interpretation in the case of this fraction. No other data are available however by which to identify the substance or compound concerned.

The difference in behavior of the fractions of complement when heated separately and when heated together suggests that during the process of thermoinactivation the ions of the euglobulin compound combine or interact chemically with substances contained in the pseudoglobulin and albumin fraction.

The protection against destruction afforded by the presence of NaCl on the alkaline side of pH 6.1 to 6.4 may be explained by the depression in ionization of the Na euglobulin compound existing

[16] Rona, P., and Michaelis, L., *Biochem Z.*, 1910, xxviii, 193.
[17] Coulter, C. B., *J. Gen. Physiol.* 1920–21, iii, 309.
[18] Unpublished experiments.
[19] Personal suggestion.

at these reactions[20] which is caused by the high concentration of the common Na ion. On the acid side of pH 6.1 to 6.4 the protein exists in amounts increasing with the acidity in the cationic condition. We should expect a similar depression in the ionization of the protein in this form to be caused by the excess of the common Cl ion, but since at these reactions the destruction of complement is as great in the presence as in the absence of salt it is apparent that the behavior of the protein as cation is different from that as anion. This conclusion was suggested by Brooks[12] from a somewhat different evaluation of similar data.

CONCLUSIONS.

1. The destruction which complement undergoes on being heated in dilution in distilled water is least at a reaction between pH 6.1 and 6.4. This depends upon the relative preservation of the mid-piece function at this point. This reaction represents probably the isoelectric point of a compound of the euglobulin with some substance present also in serum.

2. During the process of thermoinactivation it is chiefly or entirely the ions of this euglobulin compound which react, and these combine or interact with substances contained in the pseudoglobulin and albumin fraction.

3. The behavior of the euglobulin is different in the anionic and in the cationic condition, since on the acid side of pH 6.1 to 6.4 the destruction by heat increases as rapidly with the acidity in the presence as in the absence of NaCl. On the alkaline side of this point the presence of NaCl protects complement from destruction because of the depression in the ionization of the euglobulin.

[20] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 39, 237, 363, 483, 559.

A SIMPLE CASE OF SALT ANTAGONISM IN STARFISH EGGS.

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The freshly laid egg of the common starfish, *Asterias forbesi*, is surrounded by a thin layer of water-swollen or gelatinous material, of about 15 to 20 microns in diameter (one-eighth to one-twelfth the egg diameter of about 160 microns), invisible to ordinary observation, but readily demonstrated by mounting the eggs in a somewhat thick suspension of India ink in sea water and examining on a slide under a moderate magnification. Each egg then appears surrounded by a clear halo outlining the space between the egg surface and the outer limit of the jelly which is impermeable to the ink particles. It is present in all full sized eggs, both mature and immature, but is absent in the smaller ovarian eggs of two-thirds or less the full diameter; evidently the water-swollen material of which it consists is separated from the egg during the later period of ovarian growth. In the starfish egg the jelly is remarkably resistant to solution in ordinary sea water and is not removed by repeated washing in this medium, even with the aid of centrifuging; in this respect the *Asterias* egg differs from the *Arbacia* egg which is surrounded by a jelly otherwise similar but readily removed by washing in sea water. In unfertilized starfish eggs the jelly shows no change after 24 hours in sea water; but it gradually disappears in fertilized cleaving eggs, possibly in consequence of increased separation of CO_2 from the eggs, since, as Garrey[1] has shown, it combines with acids and in so doing acquires increased swelling properties.

[1] Garrey, W. E., *Biol. Bull.*, 1919, xxxvii, 287.

The jelly is readily and rapidly removed from the eggs by washing in pure isotonic NaCl solution (0.54 M). This is most conveniently demonstrated as follows. Sea water containing a suspension of the eggs is gently centrifuged with a hand centrifuge, with ten or twelve slow turns, so as to collect the eggs in the narrow end of the tube; the sea water is poured off and replaced by pure 0.54 M NaCl; the tube is then inverted to suspend the eggs and again slowly centrifuged as before; the solution is poured off and replaced with fresh, and this process is repeated. The eggs are thus exposed to the pure NaCl solution free from all but traces of sea water. If they are then returned to sea water and examined in India ink, the jelly is found to have completely disappeared. There is no evidence of further secretion of jelly by eggs which have thus been freed from it and returned to sea water.

Removal of the jelly leaves the power of fertilization and development unimpaired. Thus, in a typical experiment unfertilized eggs, removed from the animal 45 minutes previously, were exposed for 6 minutes to 0.54 M NaCl with two changes of the solution and centrifuging as above. Examination in India ink showed complete removal of the jelly from all eggs. Sperm was added to part of the eggs 18 minutes after the return to sea water. A majority next day had formed blastulæ; abnormalities were, however, more frequent than in the untreated control eggs and about 20 per cent had died without development; this injurious effect is to be referred to the action of the pure unbalanced solution upon the eggs. Similarly, eggs freed from jelly subsequently to fertilization continued development and formed blastulæ, although showing also a larger proportion of abnormalities than the control.

Arbacia eggs treated as above with 0.54 M NaCl show a similar disappearance of the jelly, but the same effect is seen in sea water and in solutions of NaCl containing $CaCl_2$. In this species the jelly is more soluble than in *Asterias* and the difference between pure and calcium-containing NaCl solutions is not shown by the above method. This difference in the properties of the jelly layer in the two species indicates a specific difference of chemical composition, but so far I have made no attempt to investigate this difference in detail.

In both *Asterias* and *Arbacia* exposure of the eggs to the pure NaCl solution for a few minutes alters the consistency of the cell surface, and apparently also the permeability of the plasma membrane, in a highly characteristic manner. The eggs cohere in small clumps, or agglutinate; this effect is well marked even in eggs exposed to the pure NaCl solution in a finger-bowl without any centrifuging, but the latter process promotes the formation of larger and firmer aggregates. This agglutination, although accompanying the removal of the jelly, is an entirely independent process, and like the removal of the jelly is prevented by the presence of calcium in the solution. A further characteristic effect produced by the pure NaCl solution in *Asterias* eggs (but not in *Arbacia*) is the formation of apparently normal fertilization membranes in a certain proportion of eggs; and in some cases cleavage and development to a blastula stage result.[2] This membrane-forming and activating effect is also antagonized by calcium.[3]

The solution of the jelly in pure NaCl solution is a somewhat gradual process; the jelly gradually incorporates water, and swells, eventually losing coherence and passing into solution. The process of swelling may be arrested at any stage by returning to sea water; but there is no evidence of reversal in the sense of a return of the jelly to its original water content. Eggs were placed in a finger-bowl, the sea water removed as far as possible, and to the remaining mass of eggs (about 1 cc.) 100 cc. of 0.54 NaCl were added. From the NaCl solution eggs were returned to finger-bowls containing sea

[2] Lillie, R. S., *Am. J. Physiol.*, 1910, xxvi, 106; cf. 119.

[3] Lillie, R. S., *Am. J. Physiol.*, 1910–11, xxvii, 289. A further interesting effect of the pure isotonic NaCl solution, also antagonized by $CaCl_2$, is that it prevents the dissolution of the germinal vesicle, and hence the maturation process, in starfish eggs placed in the solution immediately after removal from the animal. If such eggs are left in the pure NaCl solution for from 5 to 10 minutes, and are then returned to sea water, they remain permanently immature. Similar treatment with a calcium-containing NaCl solution (*e.g.*, 95 volumes of M/2 NaCl plus 5 volumes of M/2 $CaCl_2$) leaves them apparently unaffected. The pure NaCl solution thus produces the same effect as weak fatty acid solution or high temperature, either of which, if applied at this time, also prevents maturation (cf. Lillie, R. S., *Biol. Bull.*, 1917, xxxii, 135). Mineral acids appear to have a similar effect (cf. Loeb, J., *Arch. ges. Physiol.*, 1902, xciii, 59).

water at the following intervals: $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, 6, 8, and 12 minutes; after 20 to 30 minutes in sea water, eggs from each bowl of the series were examined on a slide in India ink suspension. It was found that the jelly still remained about eggs that had been exposed to the NaCl solution for so long as 3 or 4 minutes, but that by that time it was swollen to several times its original volume, so that the boundary of the ink suspension was often separated from the egg by a full egg diameter. In eggs exposed to NaCl solution for 6 minutes and examined under the same conditions the jelly had completely disappeared from a large proportion of eggs, though still remaining in a greatly swollen form in most. After 12 minutes exposure the ink was in nearly all cases in contact with the egg surface, indicating complete removal of the jelly.

The removal of the jelly in NaCl solution is completely prevented by the addition of a small proportion of $CaCl_2$. The agglutinating and membrane-forming effects above described are at the same time prevented or greatly decreased. Since these two latter effects are dependent upon alteration of the surface layer of the living protoplasm, this parallelism suggests that the calcium prevents the physiological action of the pure NaCl solution through some characteristic influence on the solubility or hydration of certain compounds which are present in the protoplasmic surface layer and upon which the normal properties of this layer depend.

The following typical experiment (June 18, 1920) will illustrate. Starfish eggs were removed from the animal at 3.06 p.m.; the eggs were normal; all showed the typical jelly envelope in India ink suspension; more than 90 per cent underwent maturation and upon fertilization developed to larval stages. At 3.48 p.m. the two tubes of a hand centrifuge were filled with a suspension of unfertilized eggs in sea water and gently centrifuged until the eggs were settled in a mass about one-third of an inch deep in the narrow part of each tube. The sea water was then poured off and replaced in one tube by pure 0.54 M NaCl (lot A), in the other by a mixture of 250 cc. 0.54 M NaCl plus 15 cc. M/2 $CaCl_2$ (lot B). The solutions were added at 3.49 p.m.; the tubes were then gently centrifuged to settle the eggs and the solution was changed; this procedure was repeated. The eggs were then returned to sea water. The total time

of exposure to the solutions was 6 minutes. Examination in India ink suspension about 15 minutes later showed complete removal of the jelly in the eggs of lot A, of which about 25 to 30 per cent had also formed fertilization membranes. In the eggs of lot B the jelly was unaltered and no fertilization membranes were formed. Part of the eggs of both lots were fertilized with sperm at 4.13 p.m.; the next day about 70 per cent of the eggs of lot A had formed blastulæ, largely abnormal, while almost all of lot B had developed normally.

Other experiments of the same kind give similar results. Exposure to pure NaCl solution for some minutes removes the jelly; it also causes agglutination and injures the eggs, as shown by a certain degree of impairment and abnormality in development, and forms fertilization membranes in a considerable proportion of eggs. All of these effects are prevented by the addition of a little $CaCl_2$ to the solution.

The antagonistic action of calcium is shown in much more dilute solution than the above; *e.g.*, in M/400 $CaCl_2$ (199 volumes of 0.54 M NaCl plus 1 volume of M/2 $CaCl_2$), with the same treatment as above, the jelly remained intact. Further experiments to determine the limit of the effective concentrations were not tried. Magnesium chloride has a similar action in preventing the solution of the jelly, but is less effective.

The question of whether calcium can be replaced in this relation by other polyvalent metals has not yet been experimentally tested except in the case of aluminium. Eggs were placed in pure 0.54 M NaCl (lot A), and in 99 volumes of 0.54 M NaCl plus 1 volume of M/2 $AlCl_3$ (lot B); the solutions were changed twice by centrifuging and decanting as before. On return to sea water after 6 minutes in the solutions the eggs of lot A were found free from jelly and largely agglutinated, while those of lot B retained the jelly, although this was somewhat thinner than in the untreated control eggs; there was no agglutination. The probability is that many other polyvalent cations would be found to have this effect, as in other cases of antagonism.

THEORY.

In the present case of salt antagonism the effect, removal of the jelly, is a direct result of the incorporation of water in pure NaCl solution with consequent swelling until coherence is lost; in the same solution containing a little calcium the jelly retains its normal hydration and remains coherent and water-insoluble. The chemical composition of the jelly is unknown, but the presence of a certain proportion of protein is probable; this is suggested by its acid-combining properties,[1] also by its mode of formation as a cellular secretion, its physical consistency and its variation in properties from species to species. At least it seems clear that some substance is present forming compounds (salts) with sodium and calcium, which differ in their solubilities and in their affinities for water.

Apparently, in the presence of an excess of NaCl, a Na salt with a marked affinity for water is formed; hence the swelling and eventual solution. When $CaCl_2$ is present in the solution a water-insoluble Ca salt is formed; in the presence of a certain proportion of this the jelly as a whole remains insoluble. In other words, the normal coherence and water-insolubility of the jelly depend on the presence of a water-insoluble compound which pervades the system and renders it coherent and water-insoluble. Hence the removal of this by replacement of Ca with Na renders the whole structure soluble.

Antagonisms depending on differences in the solubilities of Na salts (or in general of alkali metal) and Ca salts (or alkali earth and heavy metal) are well known in purely physical systems. In many organic acids the Na salts are highly soluble in water, while the Ca salts are insoluble. The case of soaps is probably the most relevant to the conditions in biological systems. The physicochemical antagonisms recently investigated by Clowes,[4] using salt solutions which were allowed to drop from a stalagmometer through oil containing some fatty acid, depend on the differences between the solubilities of Na soaps and Ca soaps in the oil and the water phases respectively. The antagonisms between the influence of Na and Ca salts on the precipitation of lecithin[5] and heat-denatured egg white[6] are prob-

[4] Clowes, G. H. A., *J. Physical Chem.*, 1916, xx, 407.
[5] Koch, W., *Z. physiol. Chem.*, 1909, lxiii, 432.
[6] Mathews, A. P., *Am. J. Physiol.*, 1905, xiv, 203.

ably in part determined by similar conditions, although factors peculiar to suspensoid systems no doubt also enter in these cases. The properties of soaps, which are at once soluble in water and in many organic solvents, and which are consequently highly surface-active at water lipin interfaces, are probably of fundamental importance in protoplasmic activities. It is noteworthy that the water-combining properties of tissues and cells are influenced by salts in a manner consistent with the hypothesis that soaps or compounds with similar solubilities determine the manner in which the bound water (Overton's "Quellungswasser")[7] is held in the protoplasm. Many years ago Loeb[8] called attention to the parallel between the absorption of water by muscle immersed in isotonic solutions of different salts of alkali and alkali earth metals, and by the soaps of the same metals; thus, the muscle absorbed much more water in isotonic KCl solution than in NaCl, while in isotonic $CaCl_2$ it lost water; when the corresponding soaps are immersed in water a similar order of relative absorption is seen, K soaps (soft soaps) swelling more rapidly than Na soaps, while the water-insoluble Ca soaps do not take up water. Swelling and loss of consistency or turgor in plant tissues immersed in pure NaCl solution are well known phenomena, which have recently been investigated in great detail by Hansteen;[9] the disintegration and loosening of the intercellular coherence which he describes as occurring under these conditions are prevented by the presence of small quantities of calcium salts. Hansteen calls attention to the earlier work of Mangin[10] on the rôle of the pectin compounds in the interstitial substance or middle lamella of plant tissues; the cementing properties of this layer are, according to Mangin, dependent on the presence of an insoluble Ca compound which he calls "Ca pectinate." In the presence of NaCl solution free from Ca this layer becomes soluble and absorbs water and the cells fall apart. Herbst's[11] observations on the loss of coherence of of the blastomeres of sea-urchin eggs in Ca-free sea water probably

[7] Overton, E., *Arch. ges. Physiol.*, 1902, xcii, 115.
[8] Loeb, J., *Arch. ges. Physiol.*, 1899, lxxv, 303.
[9] Hansteen, B., *Jahrb. wiss. Bot.*, 1910, xlvii, 289; 1913–14, liii, 536.
[10] Mangin, L., *J. Bot.*, 1893 (cited from Hansteen).
[11] Herbst, C., *Arch. Entwcklngsmechn.*, 1900, ix, 424.

have a similar general significance. The coherence of the ciliated cells in the gill epithelium of mollusca (Mitylus) is similarly lost in pure isotonic solutions of many Na salts, in which also the cells absorb large quantities of water;[12] these effects are prevented by the addition of Ca to the solution. The action of pure NaCl solutions in increasing the permeability of plant tissues to ions,[13] and of structures like the membrane of the *Fundulus* egg[14] to salts and water, is closely related to the above; similarly with the breakdown of protoplasmic structures like cilia and plasma membranes in this solution;[15] here the structural continuity is lost and with it the dependent properties of coherence and semipermeability. In all of these cases an essential part of the protective or antitoxic action of the calcium consists in preventing physical disintegrations of this kind; such disintegrations are apparently the direct result of replacing solid water-insoluble material and structure by water-soluble.

The difference between the water-combining powers of Na and Ca proteinates has been pointed out recently by Loeb in a series of papers on the influence of inorganic salts on the physical properties of proteins;[16] here also characteristic salt antagonisms affecting physical properties such as viscosity, swelling, osmotic pressure, precipitability by alcohol, are observed when the Na and Ca salts are present in certain proportions.[17] It seems probable that certain types of biological salt-antagonisms are to be explained by reference to general facts of this kind; the case of the *Fundulus* egg, where the toxic action of the pure NaCl solution is associated with a destruction of the water-proof character of the membrane or chorion enclosing the egg,[14] appears to exemplify this condition. In the case of antagonisms in living protoplasm, however, not only the proteins but other compounds, and especially the lipoids, appear to be essentially concerned. The salt antagonisms studied by Clowes,[4] which

[12] Lillie, R. S., *Am. J. Physiol.*, 1906–07, xvii, 89.

[13] Osterhout, W. J. V., *Science*, 1912, xxxv, 112; xxxvi, 350.

[14] Loeb, J., *Science*, 1912, xxxvi, 637; *Biochem. Z.*, 1912, xlvii, 127.

[15] Lillie, R. S., *Am. J. Physiol.*, 1903–04, x, 419; 1909, xxiv, 23; 1912–13, xxxi, 259.

[16] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343; 1918, xxxiii, 531; 1918, xxxiv, 77.

[17] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 3; 1918, xxxiv, 395; 489; and xxxv, 497.

exhibit such a remarkable parallelism with physiological antagonisms, are referable to the formation of soaps or soap-like compounds. It seems probable that variations in the partition of such compounds between the aqueous and the non-aqueous or organic solvents of the protoplasm are important factors in the physiological effects produced by varying the proportion of the inorganic salts in the medium. Clowes attributes the variations in the drop numbers of the solutions flowing through the oil from the stalagmometer to variations in the ratio of the oil-soluble to the water-souble soaps. These soaps have opposite influences on the surface tension factor opposing the detachment of the drop. Oil-soluble compounds like higher alcohols influence the drop numbers in the same manner as Ca salts. The recent observations of Heilbrunn[18] on changes in protoplasmic consistency under the influence of lipoid-solvent compounds also suggest that variations in the partition of compounds between the aqueous and the lipoid phases of protoplasm may influence greatly the type of protoplasmic consistency or structure.

In general, it may be inferred from the above facts that the presence of a certain proportion of solid water-insoluble salt-like compounds in the protoplasmic surface film is necessary to the normal semipermeability and structural permanence of this structure; similar considerations apply to filamentous solid structures like cilia and to the other solid structural elements of the cell. This view explains the destructive action of pure solutions of alkali salts like NaCl, which have the effect of substituting water-soluble Na-compounds for the water-insoluble Ca compounds (*e.g.*, soaps) normally present. Hansteen's[9] results with plant tissues afford strong evidence that water-insoluble Ca compounds are essential to the stability of the normal protoplasmic structures, and also of cell walls and similar structures formed by protoplasmic activity. He attributes great importance to lipoid compounds present in the protoplasmic surface layers and in the cell walls, and especially to the formation of water-insoluble combinations between these materials (together with pectin) and calcium. He regards these calcium compounds as necessary for the normal coherence of cells, as shown by their presence

[18] Heilbrunn, L., *Biol. Bull.*, 1920, xxxix, 307.

in the middle lamella, and also for the normal properties of the surface protoplasm.[19] According to Hansteen, pure alkali salt solutions attack and alter primarily the lipoid constituents of the cell walls,[20] inducing absorption of water and consequent disintegration; and he cites especially the work of Krefting,[20] who obtained from the intercellular substance of brown algæ an acid material ("Tangsäure") which forms water-soluble salts with alkali metals and water-insoluble salts with alkali earths (Ca, Sr, Ba). This compound apparently corresponds with the Ca pectinate to which Mangin attributed the cohesive properties of the material composing the middle lamella.

Hansteen's further observation that the presence of Ca salts in culture solutions is highly favorable to the branching of the roots and the growth of root-hairs in seedlings also indicates that Ca compounds are necessary to the formation of the solid structures essential to normal growth. For example, the development of root-hairs is greatly promoted in media containing several times the normal concentration of Ca salts, a result which has recently been confirmed by Wiechmann[21] in Höber's laboratory. Wiechmann finds also that strontium, barium and certain heavy metals (Mn, Ni, Co) act similarly to calcium, while magnesium is ineffective. Such data, when considered in relation to those cited above, throw an interesting light on the general significance of calcium in the formation of organic structure. Certain properties of the living protoplasmic structures, such as rigidity, water-insolubility and impermeability to water-soluble substances, seem to require the presence of calcium compounds.[22] The rapid alteration of the superficial protoplasmic

[19] Hansteen, B., *Jahrb. wiss. Bot.*, 1910, xlvii, 374.

[20] Hansteen, B., *Jahrb. wiss. Bot.*, 1913–14, liii, 574.

[21] Wiechmann, E., *Arch. ges. Physiol.*, 1920, clxxxii, 99.

[22] The general view that the presence of water-insoluble materials in the plasma membranes (formed from inorganic salts present) is responsible for their peculiar osmotic properties is by no means a new one, having apparently first been suggested by Traube in 1867 on the basis of his work with precipitation membranes. Recently the subject has been reviewed by Meigs (Meigs, E. B., *Am. J. Physiol.*, 1915, xxxviii, 456), who has studied the effects of impregnating colloidal membranes with various inorganic precipitates. He finds that membranes made by precipitating Ca and Mg phosphates in thin sheets of celloidin show

layer or plasma membrane in pure solutions of Na salts is of a kind which is consistent with this interpretation, since the essential feature of the effect produced is an increase of permeability, allowing the ready penetration of water-soluble substances (sugars, neutral salts, etc.) to which previously the membrane formed a complete barrier.[23]

SUMMARY.

The jelly surrounding the eggs of the starfish, *Asterias forbesi*, is insoluble in normal sea water, but rapidly swells and dissolves when the eggs are washed in a pure isotonic solution of NaCl. In the presence of a small proportion of $CaCl_2$ this solvent and disintegrative action of the NaCl solution is entirely prevented, and in the mixed solution the jelly exhibits the same insolubility and other properties as in normal sea water.

2. This action of $CaCl_2$ in preventing the dissolution of the jelly runs parallel with its action in preventing certain definite effects

many resemblances to plasma membranes in their osmotic properties, especially in their slight permeability to soluble inorganic salts and sugar, and suggests that these phosphates may play a part in determining the properties of the membranes of cells. This possibility should not be overlooked, although the above evidence indicates that insoluble salts formed with the colloidal compounds of the cell are of chief importance.

[23] Apparently the biologically essential properties of an organic membrane are referable chiefly to those of the limiting surface layers. Hence the cases of salt antagonisms at metallic and other surfaces have also an intimate bearing on the present problem. Bredig and Weinmayr (*Z. physik. Chem.*, 1903, xlii, 601) cite cases of such influence in the Hg–H_2O_2 catalysis; thus KOH and KCl may act as antagonists; the rhythm is extinguished by adding KCl and is restored by KOH. Related phenomena are seen in the influence of cations of high oxidation potential, like Ag, in preventing the spontaneous activation of passive iron in solutions of $NaNO_3$ and similar salts (Lillie, R. S., *Science*, 1919, 1, 416). Evidently the permeability and electromotor properties of any interfacial film depend on its composition and physical condition, both of which vary with the internal composition of the two adjoining phases. Both physical and chemical factors are concerned in the formation of the plasma membrane; adsorption, by which (presumably) the surface materials are assembled, is usually classed as a physical process; but the special composition of the membrane depends on the specific character of the cell metabolism, and on the nature of the reactions (oxidations, etc.), occurring at the cell surface.

of the pure NaCl solution on the living egg (agglutination, cytolytic action, membrane formation, prevention of maturation).

3. The inference is that the essential factor in these and similar antagonistic and protective actions is the formation of solid water-insoluble colloidal salts (*e.g.*, soaps and proteinates) of calcium (or other metal) with the structural colloids of the protoplasm. Apparently the presence of a certain proportion of such compounds is necessary to the structural stability of the living protoplasm, and especially to the water-insolubility and semipermeability of its external layer or plasma membrane. When the cell is immersed in the pure NaCl solution, water-soluble Na compounds are substituted for the insoluble Ca compounds which normally provide the necessary insolubility and coherence, and disintegration results.

STUDIES IN WOOD DECAY.

II. Enzyme Action in Polyporus Volvatus Peck and Fomes Igniarius (L.) Gillet.

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(Received for publication, April 25, 1921.)

The present paper is the third of a series,[1,2] of papers dealing with the enzyme action in the wood-destroying fungi. It is the second of a series[3] of papers to be issued by this Laboratory dealing with the decay of wood in the broadest sense.

From the standpoint of parasitism, *Polyporus volvatus* is one of the most interesting of the wood-destroying fungi. Although no inoculation experiments have been made, numerous observations tend to confirm the opinion of the writer that *Polyporus volvatus* is truly parasitic. Throughout Washington, Oregon, and Idaho it is not at all unusual to find fruiting bodies of *Polyporus volvatus* appearing in great numbers over practically the entire surface of the trunk of Douglas fir, white fir, and western hemlock. This condition may be observed on trees still having a green, healthy foliage as well as on trees which to all appearances have been killed by the fungus.

[1] Schmitz, H., and Zeller, S. M., Studies in the physiology of the fungi IX. Enzyme action in *Armillaria mellea* Vahl., *Daedalea confragosa* (Bolt.) Fr., and *Polyporus lucidus* (Leys.) Fr., *Ann. Missouri Bot. Garden*, 1919, vi, 193–200.

[2] Schmitz, H., Enzyme action in *Echinodontium tinctorium* Ellis and Everhardt, *J. Gen. Physiol.*, 1919–20, ii, 613.

[3] Schmitz, H., and Daniels, A. S., Studies in wood decay I. Laboratory tests on the relative durability of some western coniferous woods with particular reference to those growing in Idaho, School of Forestry, Univ. Idaho, Bull. 1, 1921, 1–12.

The fact that *Polyporus volvatus* may be parasitic seems to have been first suggested by Zeller[4] in an unpublished paper. Dr. Zeller writes in part, "The fact that there were still needles on the last year's growth and that the mycelium had spread from the base of the tree up the trunk for 45 to 50 feet indicates that the fungus is parasitic. The writer has no absolute proof of this statement."

In this region *Fomes igniarius* is the cause of a serious white heart rot in the common aspen (*Populus tremuloides*) and also causes a a similar heart rot in mountain birch (*Betula fontinalis*). Quite recently Weir[5] has reduced *Fomes igniarius* and *Fomes nigricans* Fr. to synonomy.

Thus, the two fungi considered here are not without considerable interest both from the general and economic point of view.

Methods.

The cultures of the fungi used in the present study were obtained from young sporophores by the tissue method. As before, the cultures were grown on sterile carrots and while still in an active growing condition, removed from the flasks, dried, and ground. All of the enzyme cultures were set up in duplicate and 0.25 gm. of fungous meal was invariably used. Otherwise, the methods followed are those previously described.

Esterases.

The esterase activity of *Polyporus volvatus* and *Fomes igniarius* was studied by the use of 1 per cent solutions of methyl acetate, ethyl acetate, ethyl butyrate, triacetin, and olive oil emulsion. After twenty-one days incubation, hydrogen ion concentration determinations were made of the various filtrates. Positive esterase activity was obtained in the case of both fungi when methyl acetate was used as the substrate. The action on all the other substrates was negative.

[4] Zeller, S. M., Wood destroying fungi of Washington. Unpublished paper.

[5] Weir, J. R., Some observations on abortive sporophores of wood destroying fungi. *Phytopathology*, 1915, v, 48–50.

Carbohydrases.

The action of carbohydrases was determined on 1 per cent solutions of maltose, lactose, sucrose, raffinose, potato starch, inulin, white fir cellulose, and hemicellulose from date seed endosperms. After varying periods of incubation, the cultures were filtered and 5 cc. samples of the filtrates treated with 20 cc. of Fehling's solution. The results in the following table are the average of two titrations and are expressed as the number of cubic centimeters of 0.05 N potassium permanganate solution required to oxidize the dissolved copper.

TABLE I.

Carbohydrase Action of Polyporus volvatus and Fomes igniarius.

Incubation period.	Substrate.	*Polyporus volvatus.*			*Fomes igniarius.*	
		With fungous meal.	With fungous meal autoclaved.	Without fungous meal.	With fungous meal.	With fungous meal autoclaved.
		0.05 N $KMnO_4$				
days		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
14	Maltose	21.85	14.70	13.25	30.40	17.15
20	Lactose	26.65	21.40	18.30	29.60	22.00
3	Sucrose	4.35	3.65	0.75	13.10	6.00
4	Raffinose	8.80	3.55	0.10	11.50	4.35
4	Potato starch	19.00	2.75	0.10	8.10	3.55
10	Inulin	5.00	3.10	0.15	7.50	5.00
30	White fir cellulose	6.00	2.85	0.20	8.00	4.95
30	Hemicellulose	7.80	3.45	1.80	8.70	5.25

Glucosidase.

The presence of a glucosidase in the fungous meal of the two fungi here discussed was determined by the action of the fungous meal upon a 1 per cent solution of salicin. After a 5 day incubation period, the enzyme cultures were filtered and sugar determinations made of 5 cubic centimeter samples of the filtrates. The results, expressed in the number of cubic centimeters of 0.05 N potassium permanganate solution required to oxidize the dissolved copper are shown in Table II.

Tannase.

Under the action of tannase, digallic acid is probably converted into gallic acid in accordance with the general formula,

$$C_{14}H_{10}O_9 - H_2O = 2C_6H_2(OH)_3COOH$$

Whether or not this reaction took place was determined by titrating the filtrates against 0.05 N iodine after the digallic acid had been precipitated out by the addition of albumin and the albumin salted out with NaCl. As indicated in Table III, the results are negative.

TABLE II.

Glucosidase Action of Polyporus volvatus and Fomes igniarius.

	Polyporus volvatus.	*Fomes igniarius.*
	0.05 N $KMnO_4$	
	cc.	cc.
1 per cent salicin + fungous meal	11.10	15.15
1 " " " + " " autoclaved	3.50	4.50
1 " " "	0.40	0.40

TABLE III.

Tannase Action of Polyporus vovatus and Fomes igniarius.

Enzyme Culture.	*Polyporus volvatus.*	*Fomes igniarius.*
	0.05 N iodine.	
	cc.	cc.
1 per cent digallic acid + fungous meal	4.7	3.9
1 " " " " + " " autoclaved	4.7	3.9
1 " " " "	3.5	3.5

The absence of tannase in *Polyporus volvatus* is difficult to account for since the fungus inhabits the barks of trees having a high tannin content. Its absence may, however, be due to the fact that the culture medium upon which it had been grown contained little or no tannin. The influence of the culture medium on the production of tannase in fungi has been fully investigated by Knudson.[6]

[6] Knudson, L., Tannic acid fermentation. I, *J. Biol. Chem.*, 1913, xiv, 159, Figs. 1 and 2; Tannic acid fermentation. II, 185.

Urease and Amidase.

To determine the presence or absence of enzymes which split amino-acids and urea into ammonia and hydroxy acids, the indicator method suggested by Schmitz and Zeller[1] was employed. The results are shown in Table IV.

TABLE IV.

Urease and Amidase Activity of Polyporus volvatus and Fomes igniarius.

	Enzyme culture.		Approximate change in hydrogen ion concentration.	
			Urea.	Acetamid.
Polyporus volvatus.	Substrate + fungous meal.	1	*pH* 5.6–6.0 3 min.	5.6–5.6 3 min.
		2	5.6–6.0 3 min.	5.6–5.6 3 min.
	Substrate + fungous meal autoclaved.	3	5.6–6.0 3 min.	5.6–5.6 3 min.
		4	5.6–6.0 3 min.	5.6–5.6 3 min.
Fomes igniarius.	Substrate + fungous meal.	1	5.6–7.8 45 sec.	5.6–5.6 3 min.
		2	5.6–7.8 45 sec.	5.6–5.6 3 min.
	Substrate + fungous meal autoclaved.	3	5.6–6.0 3 min.	5.6–5.6 3 min.
		4	5.6–6.0 3 min.	5.6–5.6 3 min.
Control.	Substrate alone.	1	5.6–6.0 3 min.	5.6–5.6 3 min.
		2	5.6–6.0 3 min.	5.6–5.6 3 min.

Positive results were obtained only in the case of *Fomes igniarius* when urea was used as a substrate.

Rennet.

When 0.25 gm. of fungous meal was added to 10 cc. of fresh milk, coagulation occured in 3 hours in the case of *Polyporus volvatus*, and in 4½ hours in the case of *Fomes igniarius*.

Catalase.

The presence of catalase was demonstrated by the action of the fungous meal upon a 3 per cent solution of hydrogen peroxide. When 0.25 gm. of the meal was added to 100 cc. of solution, 9.5 cc. of oxygen were liberated in the case of *Polyporus volvatus*, and 12 cc. in the case of *Fomes igniarius* in a period of 5 minutes.

Protease.

Tryptic and ereptic fermentation was studied by the use of albumin, peptone, casein, and fibrin in enzyme cultures having a neutral acid and alkaline reaction. The biuret test was employed in testing for the action of erepsin. The enzyme cultures were filtered, crystalline ammonium sulphate was added to the filtrate to precipitate the native proteins, and the solutions were again filtered through bone-black to remove the precipitate. In no case was a pink color produced when sodium hydroxide and dilute copper sulphate were added to the second filtrate.

A tryptophane test was also made of the filtrate by adding a few drops of glacial acetic acid and a few drops of strong bromine water. In no case was a pink color produced.

SUMMARY.

Circumstantial evidence is presented which indicates that *Polyporus volvatus* is parasitic.

Cultures of *Polyporus volvatus* and *Fomes igniarius* may be obtained from the young sporophores by the tissue method.

In *Polyporus volvatus* the presence of the following enzymes was demonstrated: esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, glucosidase, rennet, and catalase.

In *Fomes igniarius* the presence of the following enzymes was demonstrated: esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, glucosidase, urease, rennet, and catalase.

STUDIES ON THE REGULATION OF OSMOTIC PRESSURE.

I. The Effect of Increasing Concentrations of Gelatin on the Conductivity of a Sodium Chloride Solution.

By WALTER W. PALMER, DANA W. ATCHLEY, and ROBERT F. LOEB.

(From the Chemical Division of the Medical Clinic of the Johns Hopkins University and Hospital.)

(Received for publication, May 20, 1921.)

INTRODUCTION.

In a study of the factors influencing the regulation of osmotic pressure of the body fluids, the physical properties have been determined in a series of blood sera from normal and pathological individuals. In the case of some patients, there was found a strikingly low percentage of serum protein. The conductivity of the salts in blood with a low percentage of serum protein was high compared with the conductivity of the same salts in normal blood. It occurred to us that possibly the protein molecules offer mechanical interference to the passage of the current through the solution, hence, when the protein content of the solution is diminished the observed conductivity will increase without a corresponding increase in the concentration of ions present. It was to throw light upon this hypothesis that a study of the relation between conductivity and concentration of gelatin was undertaken.

In 1898 Bugarszky and Tangl[1] pointed out that, of the non-conducting substances in the serum, protein is the only one present in amounts sufficient to affect the electrical conductivity. These authors derived a formula by the addition of serum protein in varying quantities to a salt solution of constant conductivity. The serum protein was obtained by dialyzing blood serum for two months and concentrating under reduced pressure. The hydrogen ion concentration was not controlled in their experiments.

[1] Bugarszky, St., and Tangl, F., *Arch. ges. Physiol.*, 1898, lxxii, 531.

EXPERIMENTAL.

Powdered gelatin was used in these experiments and was purified at the isoelectric point according to the method described by Loeb.[2] The percentage of gelatin in the final solutions was determined by drying samples to constant weight. The hydrogen ion concentration was determined by a gas chain and the conductivity measurements were made with a Kohlrausch bridge at 25°C. The physical methods were standardized by simple inorganic solutions. Duplicate observations were made in each case. The concentrations of gelatin ranged from 0.8 per cent to 6.5 per cent and the experiments were carried out at three different hydrogen ion concentrations. One series of observations was made at pH 5.1, near the isoelectric point of gelatin,

TABLE I.

Conductivity of Pure Gelatin Solutions.

Gelatin.	Conductivity $\times 10^{-4}$ at pH 3.3	Conductivity $\times 10^{-4}$ at pH 5.1	Conductivity $\times 10^{-4}$ at pH 7.4
per cent			
0.8	6.6	0.3	1.3
1.7	10.7	0.6	2.4
3.3	19.1	1.0	4.2
4.9	25.7	1.3	5.9
6.5	30.9	1.5	7.2

another at 3.3 because of the relatively high ionization of gelatin at that acidity, and finally one at pH 7.4, approximately the reaction of blood. The gelatin was brought to these hydrogen ion concentrations by the addition of HCl or NaOH.

A preliminary experiment, Table I, was carried out on pure gelatin solutions varying in concentration from 0.8 per cent to 6.5 per cent of gelatin. The conductivity of these solutions was determined at the three different hydrogen ion concentrations mentioned above, *viz.* 5.1, 7.4, and 3.3. Fig. 1 shows the concentrations of gelatin as the abscissæ and the specific conductivities $\times 10^{-4}$ as ordinates. The curves at the three reactions are reduced to the same scale and plotted at equal intervals above each other. The conductivity in-

[2] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 341.

creases with each increment of gelatin added but the curve is not a straight line in any instance. The actual change in conductivity as the concentration of gelatin increases from 0.8 per cent to 6.5 per

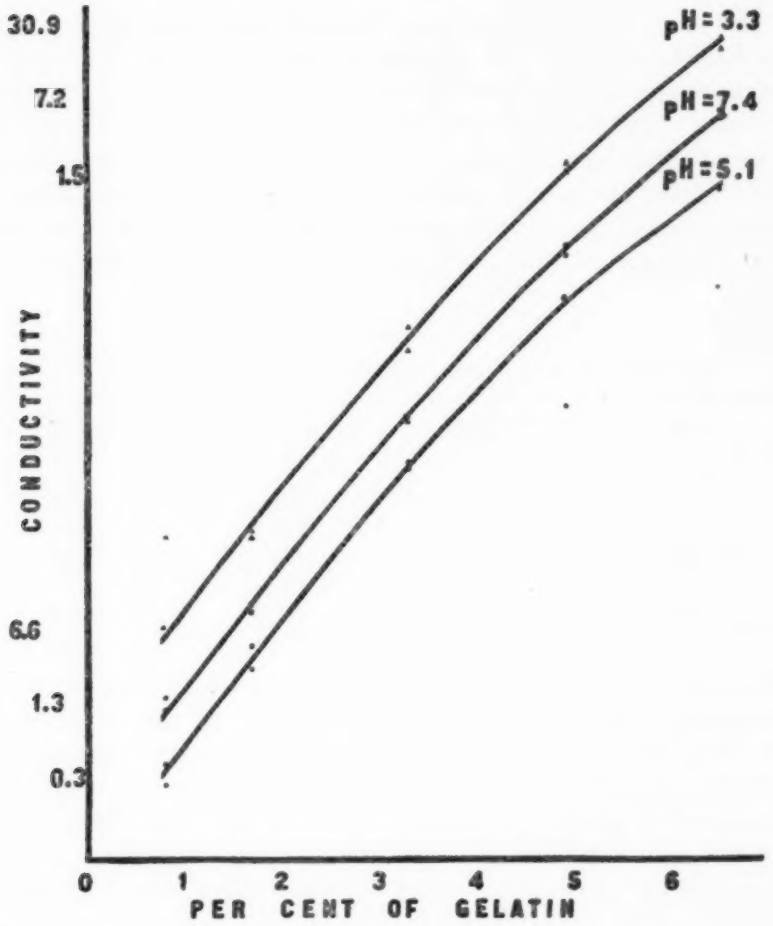

FIG. 1. Conductivities of pure gelatin solutions. The abscissæ represent varying concentrations of gelatin in per cent and the ordinates represent specific conductivities $\times 10^{-4}$. With increase in concentration of gelatin from 0.8 per cent to 6.5 per cent it is seen that the conductivity of gelatin at pH 5.1 increases from 0.3 to 1.5, at pH 7.4 it increases from 1.3 to 7.2 and at pH 3.3 it increases from 6.6 to 30.9.

cent is from 0.3 to 1.5 at pH 5.1 while at 3.3 the range is from 6.6 to 30.9. This difference in ionization with change in hydrogen ion concentration has been thoroughly studied in 1 per cent gelatin solutions by Loeb.[3]

The next series of experiments, Table II, bears more directly upon the original problem and consists of observations on the effect of gelatin upon the conductivity of a 0.6 per cent sodium chloride solution. The concentrations of gelatin and the reactions of the final solutions were the same as were used in the first experiments. Fig. 2 shows the results plotted on a common scale, conductivities again as ordinates and concentrations of gelatin in per cent as abscissæ. The curves for each pH are appropriately labeled. All three curves

TABLE II.

Conductivity of 0.6 per cent NaCl Solutions with Increasing Gelatin.

Gelatin.	Conductivity $\times 10^{-4}$ at pH 3.3	Conductivity $\times 10^{-4}$ at pH 5.1	Conductivity $\times 10^{-4}$ at pH 7.4
per cent			
0.8	114.5	108.9	109.8
1.7	115.2	106.7	108.2
3.3	117.3	102.6	105.9
4.9	119.8	98.1	104.2
6.5	121.7	94.1	101.0

are apparently straight lines. At pH 3.3 the conductivity increases consistently with the concentration of gelatin; at pH 5.1, however, the conductivity decreases strikingly with increasing amounts of gelatin. At pH 7.4 the conductivity again decreases with each increment of gelatin but the depression of the conductivity is less marked than at pH 5.1. Clearly the addition of gelatin at pH 3.3 to a solution of sodium chloride increases the conductivity of the solution; while the addition of gelatin near the isoelectric point (pH 5.1) decreases the conductivity in proportion to the amount of gelatin added. At the reaction of blood, also, the addition of gelatin to a sodium chloride solution depresses rather than increases the con-

[3] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 247.

ductivity of the resulting solutions. This latter result is evidence in favor of our original hypothesis that decreasing the percentage of protein in blood serum increases the conductivity of that serum.

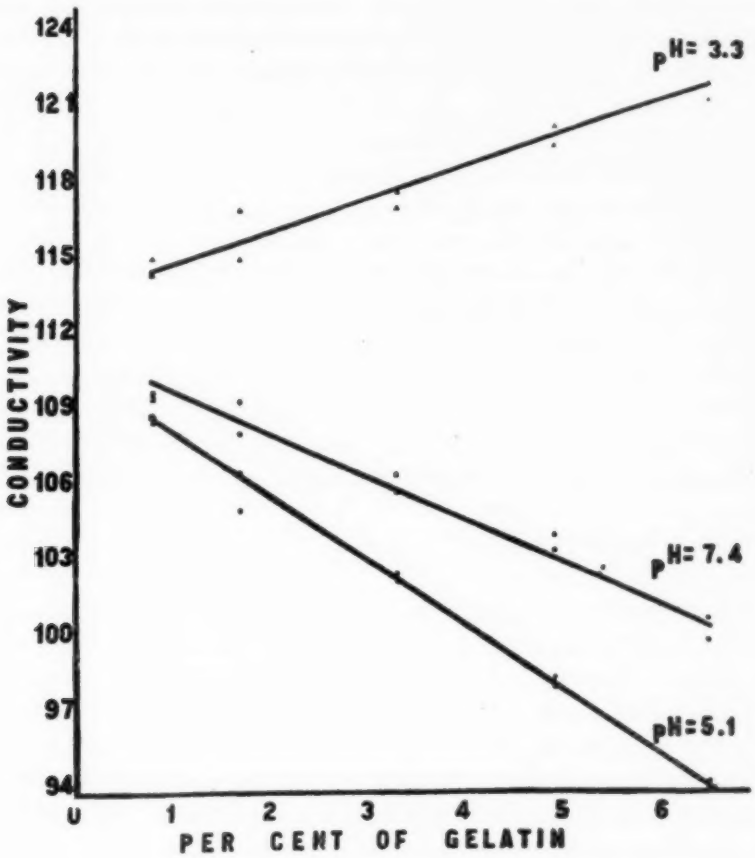

FIG. 2. Changes in conductivity of 0.6 per cent NaCl solutions with increasing concentration of gelatin (from 0.8 per cent to 6.5 per cent) at various hydrogen ion concentrations. At pH 3.3, the specific conductivity $\times 10^{-4}$ *increases* from 114.5 to 122. At pH 5.1, the conductivity *decreases* from 108.8 to 94.2. At pH 7.4 the conductivity *decreases* from 109.7 to 101.

DISCUSSION.

The results charted in Fig. 2 may be explained as follows. The addition of gelatin to a solution of sodium chloride has two possible effects, which tend to oppose each other, (1) to increase the conductivity by the addition of ionized gelatin salts and (2) to decrease the conductivity by the mechanical interference of the large undissociated gelatin molecules. It could be predicted from the newer conceptions of the physical chemistry of the proteins that the first of these effects would be considerably greater at pH 3.3 than at pH 5.1, which is near the isoelectric point of gelatin and futhermore, it would be expected that the results obtained at pH 7.4 would be more nearly like the results observed at pH 5.1 than like those observed at pH 3.3. Whether the addition of gelatin at a given hydrogen ion concentration increases the conductivity or depresses it depends, therefore, upon the degree of ionization of the gelatin at that reaction. It is obvious that at the reaction of blood, the pure protein, gelatin, is so little ionized that the mechanical interference predominates and the conductivity decreases with each increment of gelatin.

CONCLUSIONS.

1. In pure gelatin solutions the conductivity of the solution increases with increasing concentrations, regardless of the hydrogen ion concentration. The actual value of the specific conductivity is greater at that reaction where the degree of ionization is greater.

2. The addition of gelatin in increasing concentrations to a 0.6 per cent sodium chloride solution affects the conductivity of that solution in two ways: (*a*) At pH 3.3, (where gelatin is highly ionized) the conductivity increases with each added increment of gelatin. (*b*) At pH 5.1 and 7.4 (where gelatin is less highly ionized) the conductivity decreases with each added increment of gelatin.

A similar study is being made of crystalline egg albumin.

CORRELATION OF THE PROPAGATION-VELOCITY OF THE CONTRACTION-WAVE IN MUSCLE WITH THE ELECTRICAL CONDUCTIVITY OF THE SURROUNDING MEDIUM.

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(Received for publication, May 20, 1921.)

An attempt was made at Clark University in 1916 and 1917 to test experimentally the thesis that the rate of physiological conduction in irritable tissues is a direct function of the electrical conductivity of the medium in which the tissue acts. The investigation was a series of experiments with reference to the propagation-velocity of the contraction-wave in the heart muscle of the river terrapin under artificial conditions. Measurements of the velocity of the wave in a series of isotonic balanced salt solutions of graded activity (mixtures of Ringer's solution with isotonic sugar solution) were made by means of special apparatus. These preliminary results indicated a direct correlation between the propagation-velocity and the electrical conductivity, and that the ratio of velocity with respect to conductivity was nearly a constant until the concentration of the salts of the Ringer's solution was reduced more than half. The original apparatus has been modified and the investigation has been extended during 1919 and 1921 to include the heart of the king-crab, *Limulus*, and the sartorius of the leopard-frog, *Rana pipiens*. Observations have been made in the above solutions over an extended range of electrical conductivity and under nearly constant conditions of oxygen tension, hydrogen ion concentration, and temperature.[1]

Comparative observations upon the spreading of an excitation state in irritable tissues indicate that the speed of conduction is

[1] Pond, S. E., Contraction wave in heart-muscle of River Terrapin, Thesis Worcester, 1917.

dependent principally upon the specific constitution of the tissue, the temperature, and the composition of the surrounding medium. What part the medium plays in the transmission of the contraction wave is not fully known.

Mayer[2] found that the rate of nerve conduction in the marine medusa *Cassiopea* was closely proportional to the total concentration of the cations Na, Ca, and K in the medium. When the sea water was diluted with distilled water a decline in the rate of nerve conduction was observed with increasing dilution. He attributed these results to the change in the adsorption of the above cations by the tissue; but it may also indicate, as Lillie pointed out[3], a direct correlation of the rate of physiological conduction with the electrical conductivity of the medium. In fact, the decline in propagation-rate runs closely parallel with the decline in the electrical conductivity of the sea water when similarly diluted. Mayer, however, found that under some conditions the propagation-rate may be independent of the electrical conductivity; thus a dilution of sea water with 0.4 *molar* magnesium chloride solution causes a decrease in the velocity of the nerve conduction to a degree closely proportional to the degree of dilution, although the electrical conductivity remains essentially unchanged.[4] The addition of magnesium however, must disturb the balance of the salts in the medium and introduce other factors of a special kind; and the possibility remains that in physiologically balanced media the rate of transmission of the excitation state may be determined, other conditions being equal, by the electrical conductivity of the solution.

In the light of these and other facts and of general theoretical considerations, Lillie advocates the theory "that the transmission of the excitation-state from the immediate site of activity to the adjoining resting areas is dependent on an *electrical local action* of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces (*e.g.*, of iron) in con-

[2] Mayer, A. G., *Am. J. Physiol.*, 1915–16, xxxix, 375; 1916–17, xlii, 469; 1917 xliv, 591.

[3] Lillie, R. S., *Am. J. Physiol.*, 1916, xli, 133.

[4] Mayer, A. G., *Am. J. Physiol.*, 1915–16, xxxix, 381.

tact with an electrolyte solution."[5] This hypothesis "assumes that the electromotor properties of the protoplasmic surface-film are determined by conditions which are fundamentally similar to those governing the electromotor phenomena at metallic surfaces."[6] With irritable tissues, just as with metallic elements under certain conditions, *e.g.*, passive iron in nitric acid solution, local alteration gives rise to a characterisitic spreading effect, which has a rate dependent partly upon the composition of the medium, and partly upon the peculiarities of the tissue or metal.[7] The effects of local stimulation in living tissues spreads to other parts at different rates, very rapidly in some tissues, *e.g.*, nerve, while in others, *e.g.*, smooth muscle, the rate of transmission is slow. Similarly there is a wide varation in the rate of electrolytic changes in metals under different conditions.[8, 9] The slow extension of a rust spot in iron in the presence of an electrolyte is an instance of a gradual spreading effect; while the change from the passive to the active state, in iron and other metals, spreads from a region of local alteration, under certain conditions, with great rapidity.[10, 11]

The general view that the bioelectric variation, as such, is the essential change on which conduction of excitation in irritable tissues depends is by no means a new one and was favored by du Bois-Reymond, Hermann, Kühne and other early students of the bioelectric phenomena.[12]

The present investigation relates to the rôle of the composition and electrical conductivity of the medium in the transmission of the contraction-wave in muscle.

[5] Lillie, R. S., *Am. J. Physiol.*, 1916, xli, 126.

[6] Lillie,[5] p. 129.

[7] Lillie, R. S. *Am. J. Physiol.*, 1914, xxxiv, 414; 1915, xxxvii, 348.

[8] Lillie, R. S., *Scient. Monthly*, 1919, viii, 456, 552. *Science*, N. S., 1919, l, 259, 416.

[9] Lillie, R. S., and Johnston, E. N., *Biol. Bull.* 1919, xxxvi, 225.

[10] Bennett, C. W. and Burnham, W. S., *J. Phys. Chem.* 1917, xxi, 107.

[11] Lillie, R. S., *Am. J. Physiol.*, 1915, xxxvii, 348; 1916, xli, 126; *J. Gen. Physiol.*, 1920, iii, 107.

[12] du Bois-Reymond, E., *Ges. Abhandl. allg. Muskel- und Nervenphysik*, 1888, ii, 698, 733; Kühne, W., *Proc. Roy. Soc. London*, 1888, xliv, 446; *Z. Biol.*, 1888, xxiv, 383.

Method.

The velocity of the contraction-wave in muscle, *i.e.*, of the transmission of the excitation state, is measured by recording the time of the bulging of a strip of tissue at two or more points along its length as it undergoes contraction. In the present investigation the local bulging is made to operate (by a simple lever system) minute mirrors, which bring to focus upon a moving photographic film mirror-images of an illuminated slit. The muscle is either stimulated at one end by a minimal "break" induction-shock, or (as in the *Limulus* heart) it is rhythmically beating and the contraction-wave moving along the tissue operates the mirrors as it passes certain points. Each organ or strip of muscle while fixed in place for experimentation is arranged so that without otherwise changing the conditions it may be exposed to a succession of solutions of graded electrical conductivity; *e.g.*, mixtures of sea water or Ringer's solution with isotonic sugar solution in varying proportions (1:9, 2:8, 3:7, *etc.*). An electrically actuated tuning-fork of suitable frequency, with an attached mirror, is so arranged that a time curve is recorded upon the moving film above the muscle record. The solutions used with turtle and frog tissues have been corrected to, or kept at, constant temperature, about 20°C., and the hydrogen ion concentration of the solutions has been adjusted before experimentation to that of the Ringer's solution employed in the same series; *i.e.*, about pH = 6.8. Measurements of the hydrogen ion concentrations have been made by the colorimetric method of Clark and Lubs with standardized buffer solutions.[13] The electrical conductivity of the solutions in all the work has been measured at 25°C. by the method of Kohlrausch, determinations being made immediately before and after taking the muscle record.

The apparatus which has been used in this study for recording the passage of the contraction-wave is specially constructed so as to allow the external medium to be changed at will without otherwise disturbing the tissue. It is described in detail by the author elsewhere. The supporting part of the apparatus coming into direct contact with the frog and turtle tissues is entirely composed of vulcanized

[13] Clark, W. M., The determination of hydrogen ions, Baltimore, 1920, 38ff.

fiber impregnated with Bakelite; this substance is insoluble in the solutions used, and does not affect the tissues. In the experiments with the heart of *Limulus* the organ was fixed in a trough composed of paraffin.

The tissues were prepared as follows: Frogs were first curarized, with about 2 cc. of a 1 per cent solution of curare (in Ringer's solution), and after a period of 20 minutes both sartorii were removed. One of the muscles was mounted on the support and the other kept in cold Ringer's solution. The companion muscle was used in a few cases in duplicate experiments. The ventricle of the turtle was removed, cut into rings, and two of these opened up as strips of thick muscular tissue. One was placed in position on the apparatus and the other kept reserve for later use. The heart of the female *Limulus* (in preference to the male because of its greater length) was removed through a dorsal opening in the carapace and placed in the paraffin trough with the ventral side up, so that the ganglion was completely immersed in the solution. On one occasion, the muscle tissue was cut away from the ganglion between the third and sixth segments, leaving the anterior and posterior segments connected by the ganglion.

Complete records of one or more muscle contraction waves were taken upon a strip of photographic film wrapped about a kymograph drum. The procedure adopted was as follows: The tissue was placed in position for a record, washed in at least two changes of the test solution over a period of 15 or 20 minutes, and then exposed to a final bath in which the records were taken. *Limulus* hearts in the troughs were so arranged that the test solution flowed slowly over and about the tissue; while the turtle and frog tissues were exposed in tumblers holding about 250 cc. of solution. The arc-lamp was lighted and adjusted to uniform illumination of the mirrors; the tuning-fork was then started and the kymograph brought up to speed. The latter was so arranged (inside of a light-proof box) that a shutter on the arc-lamp remained open during one complete revolution of the drum. An inductorium circuit was closed (in response to a signal attached to the kymograph) shortly before the shutter was opened and broken just afterward. Thus during one revolution of the kymograph drum the tissue was stimulated and one complete muscle record was made, together with the time curve.

Thre curves, those of the tuning-fork and of the contraction-waves at two points of the muscle were thus described above one another. Experiments with the rhythmically beating heart of *Limulus* required opening the shutters by hand just before a contraction of the organ.

Determinations were made to ascertain the time which should elapse after a change of solution in order to enable the tissue to reach a uniform condition and permit duplicate records to be secured. This period, with frequent changes and stirring of the solutions in the tumblers, was rarely less than 20 minutes in the smallest frog muscle. During this time the records indicated gradual changes in the speed of the contraction-wave. After a lapse of this initial period records were taken at intervals duringan hour or more. These related to the behavior of the tissue (*a*) in the normal medium, Ringer's solution or sea water, (*b*) in a medium made by diluting the normal medium with isotonic sugar solution, and (*c*) again in the nor mal medium. The change in the behavior of the tissue on passing from the normal medium of high conductivity to a medium of lower conductivity could thus be compared with the change following the reverse transfer. The rate of recovery of propagation-velocity on returning from the medium of low conductivity to the normal medium could also be determined.

RESULTS.

Limulus.—The experiments upon the heart of the king-crab were designed to show (*a*) changes of irritability in pure isotonic sugar solutions, (*b*) the behavior in mixtures of sea water with isotonic sugar solution in various proportions, and (*c*) the behavior in sea water under the same experimental conditions over a period equivalent to that covered by the tests. The determinations of the propagation-velocity have been made over different distances and with respect to different portions of the hearts. The most consistent records are from the anterior third; while those from the posterior and anterior region, *i.e.*, covering the length of the whole organ, are of doubtful value. In all cases the temperature of the solutions employed has been adjusted to that of the sea water on the day of the experiment.

In running sea water the rate of beat of the excised heart may differ from that observed in the body before the operation. After transfer to the paraffin trough used in the tests the rate of beat at first decreases, or the heart may stop beating altogether. In the latter case hearts usually resume beating in a few minutes after slight pinching or tapping. The rate of beat was always found to increase during the next 10 minutes, and, after this interval it usually remained essentially unchanged. In some cases a decrease in the rate of beat was observed, and the amplitude of the contraction decreased, although the excised heart will frequently beat for 2 days or more. If now, after 30 minutes in sea water, the heart is placed in pure isotonic sugar solution, the beat at first slightly increases in rate and then diminishes through a period of 70 to 80 minutes and finally ceases altogether. In six hearts observed under these conditions the average time required for a complete loss of rhythmic beating was 76 minutes. Artificial stimulation, pinching or turning the heart about in the trough did not induce any regular beating. Rhythmical beating was resumed, however, as soon as sea water was allowed to flow about the heart for a few moments; the rate of beat was in some instances somewhat slow at first but soon became about the same as before exposure to the sugar solution. Two of the above hearts which had been exposed to sugar solution for a little over 2 hours recovered in sea water within 3 minutes; one heart exposed to sugar solution for $3\frac{1}{2}$ hours; recovered in a little less that 4 minutes. The three remaining hearts were returned to sea water within a few minutes after the rhythmic beating had stopped and recovered in between $2\frac{3}{4}$ and 3 minutes. Duplicate records of the speed of the contraction-wave were not secured in these experiments because of the variability of the beating in sugar. In solutions in which three-quarters of the sea water is replaced by sugar solution, *i.e.*, 25 per cent sea water or less, there is a constant decrement in the rhythmic beating and a final loss of irritability; the time required for this loss is greater than in pure sugar solution, varying from 2 to $3\frac{1}{2}$ hours. Attempts to get duplicate records of the propagation-velocity in these solutions were not successful. Some observations were made in 30 per cent sea water, but the velocity of the contraction-wave over two or three segments proved exceedingly slow and the observations showed poor agreement.

Most determinations of the propagation-velocities have accordingly been made in mixtures of sea water and sugar solution containing 40 per cent or more of the normal electrolyte content; *i.e.*, 0.4 or more sea water.

Estimates of the velocity of propagation of the contraction-wave in the *Limulus* heart based upon records taken from the two opposite ends of the organ are variable and inconsistent. Records taken from the second and eighth segments also appear in no cases to have given consistent results. If recording devices are attached to the second,

TABLE I.

Contraction-Wave in Limulus Heart. Experiment 30. Sea Water, 21°C.

Distance.	Velocity per second.	Segments.	Time.
cm.	*cm.*		*hrs.*
3.0	75	4→2	1
4.4	62	4→8	1
7.4		8→2	1
3.0	69	4→2	3
4.4	65	4→8	3
7.4		8→2	3
3.0	75	4→2	6
4.4	65	4→8	6
7.4		2→8	6
3.0	68	4→2	8
4.4	64	4→8	8
7.4		2→8	8
3.0	75	4→2	10
4.4	65	4→8	10
7.4		2→8	10

fourth and eighth segments as recorded in Table I, the records show that at times the contraction of the second segment may actually precede that of the eighth, while the intermediate segment may contract before either of the others. Hence the time elapsing between the contractions at opposite ends of the heart may be so brief as to show that the case is not one of simple transmission of a single contraction-wave from end to end of the organ. Transmission, however appears to be uniform over a short region near the middle of the heart. Records from this middle region, *i.e.*, fourth, fifth, and sixth segments, indicate that these segments contract almost simultaneously.

The majority of the records have been taken from two points situated a short distance apart in the middle region. The results are not combined in a single set of figures because the same conditions do not appear to prevail in all regions. Each experiment by itself however, indicates that a lowered electrical conductivity of the medium is always associated with a reduction in the propagation-velocity of the contraction-wave over the region under observation. It will be noted that in all of the experiments the ratios of velocity with respect to conductivity are of the same order.

Another variable feature has been encountered in the irregular behavior of the hearts of animals which were kept in captivity for more than two months at Woods Hole. After being exposed in the trough for a few hours such hearts would often reverse their direction of beat, or would become arhythmical. In the case of animals fresh from the shore and others which had been artificially fed with small fish the hearts showed regular behavior and have provided consistent records, from which Table II has been compiled.

In running sea water the propagation-velocity of a healthy heart may be regularly so high as 80 cm. per second, but this velocity is not always found if the same regions are compared. In the eight experiments reported the velocity varies from 63 to 81 cm. per second. The average velocity is in the neighborhood of 70 cm. per second. The concentrations given in the second column of Table II are expressed as the fractional part of sea water present in the mixed solutions, *i.e.*, a mixture of 8 parts sea water and 2 parts isotonic sugar solution is designated as "0.8 *Sw.*" The conductivity of the sea water is taken as unity, and the proportional conductivity of the mixtures if calculated from the experimental determinations of resistance. The velocity of the propagated disturbance is expressed in centimeters per second. These two sets of determinations are combined in the last column as the ratio of velocity v with respect to conductivity c. All of the calculations are subject to an error introduced in reading the records, varying from 5 per cent at the speed of 80 cm. per second to 2 per cent at 20 cm. per second.

A number of observations were made with reference to the aeration of the solution. *Limulus* hearts examined in mixed solutions made with sea water which had been shipped to Worcester from

TABLE II.

Contraction-Wave in Limulus Heart.

Record.	Experiment.	Solution.	Conductivity. (Sw 1).	Temperature.	Distance.	Velocity per second.	$\frac{v}{c} = k.$	Segments.
				°C.	cm.	cm.		
C	32	*Sw*	1.00	22.0	4.0	81	81	5→2
D	32	0.7*Sw*	0.66	22.0	4.0	55	83	5→2
E	32	*Sw*	1.00	22.0	4.0	83	83	5→2
H	32	0.5*Sw*	0.55	22.0	4.0	43	78	5→2
K	32	*Sw*	1.00	22.0	4.0	76	76	5→2
B	35	*Sw*	1.00	22.0	3.5	78	78	5→2
F	35	0.8*Sw*	0.77	22.0	3.5	57	74	5→2
J	35	*Sw*	1.00	22.0	3.5	73	73	5→2
B	36	*Sw*	1.00	22.0	5.0	78	78	6→2
F	36	0.5*Sw*	0.55	22.0	5.0	42	61	6→2
H	36	*Sw*	1.00	22.0	5.0	74	74	6→2
C	44	*Sw*	1.00	22.0	3.5	73	73	5→2
E	44	0.7*Sw*	0.66	22.0	3.5	42	64	5→2
G	44	*Sw*	1.00	22.0	3.5	73	73	5→2
I	44	0.7*Sw*	0.66	22.0	3.5	42	64	5→2
K	44	*Sw*	1.00	22.0	3.5	67	67	5→2
B	52	*Sw*	1.00	21.5	3.1	70	70	4→2
D	52	0.5*Sw*	0.55	21.5	3.1	35	64	4→2
F	52	*Sw*	1.00	21.5	3.1	60	60	4→2
I	52	0.7*Sw*	0.66	21.5	3.1	49	73	4→2
L	52	*Sw*	1.00	21.5	3.1	60	60	4→2
B	53	*Sw*	1.00	21.5	4.3	63	63	4→8
D	53	0.9*Sw*	0.92	21.5	4.3	60	65	4→8
F	53	*Sw*	1.00	21.5	4.3	63	63	4→8
I	53	0.7*Sw*	0.66	21.5	4.3	46	69	4→8
K	53	*Sw*	1.00	21.5	4.3	63	63	4→8
N	53	0.5*Sw*	0.55	21.5	4.3	36	65	4→8
P	53	*Sw*	1.00	21.5	4.3	60	60	4→8
B	54	*Sw*	1.00	21.5	4.0	63	63	5→2
D	54	0.8*Sw*	0.77	21.5	4.0	55	72	5→2
G	54	*Sw*	1.00	21.5	4.0	63	63	5→2
J	54	0.4*Sw*	0.42	21.5	4.0	24	58	5→2
L	54	*Sw*	1.00	21.5	4.0	63	63	5→2
O	54	0.6*Sw*	0.62	21.5	4.0	38	62	5→2
R	54	*Sw*	1.00	21.5	4.0	59	59	5→2
C	55	*Sw*	1.00	21.5	3.0	68	68	4→2
E	55	0.7*Sw*	0.66	21.5	3.0	47	71	4→2
G	55	*Sw*	1.00	21.5	3.0	68	68	4→2
I	55	0.4*Sw*	0.42	21.5	3.0	29	69	4→2
K	55	*Sw*	1.00	21.5	3.0	63	63	4→2
M	55	0.6*Sw*	0.62	21.5	3.0	44	71	4→2
P	55	*Sw*	1.00	21.5	3.0	63	63	4→2

Woods Hole, exhibited a smaller decrement in the rate of beat when air had been bubbled through the solutions than when this procedure was omitted. At Woods Hole the records showed better agreement when the behavior of the hearts was studied in freshly made mixtures or in aerated mixtures than when similar solutions were used which had stood in bottles for 2 days or more.

Frog.—More extensive studies of the relationship between the electrical conductivity of the surrounding medium and the propagation-velocity of the contraction-wave have been made with the sartorius muscle of the leopard-frog. Each muscle strip is mounted vertically on a supporting apparatus; the muscle is stimulated electrically at one end by minimal single shocks, and the contraction-wave travels thence to the other end of the tissue along the parallel fibers. The distances between the two points at which the wave has been recorded vary between 1.5 cm. and 2.4 cm. Break-shocks are sent into the tendinous end of the muscle through a platinum stimulating electrode. The temperature during the experiments has been adjusted to 20°C. In some cases reported below the oxygen tension has been increased by bubbling oxygen through the solutions. The hydrogen ion concentration of all the mixtures is nearly 6.8.

Tests made over long periods of time indicate that the sartorius muscle of the frog is very resistant to the artificial conditions imposed in the experiments. Observations on muscles immersed in Ringer's solution have been made at intervals during periods so long as 96 hours, and toward the end of this time only a slight loss in the propagation-velocity of the contraction-wave has been recorded. The amplitude of the contraction-wave appears to decrease very gradually during the first 2 days, and more rapidly during the third and fourth days. In solutions of cane-sugar the irritability of the tissue is soon lost completely; the time required with several changes of solution varies from 20 to 30 minutes. Upon return to Ringer's solution the irritability is restored within approximately 1 minute. At 20°C. the normal rate of conduction of the tissue in Ringer's solution is about 3 m. per second. In the Ringer-sugar mixtures this rate decreases in close proportionality with the reduction of the electrical conductivity until the concentration of the salts has been reduced to about one-half the normal. In the range between the

dilution with one-half reduction of salts to three-fourths reduction, the propagation-rate falls off more rapidly than the electrical conductivity; while if more of the Ringer salts be replaced by sugar the irritability is soon decreased to zero. In the solutions of low electrical conductivity the tissue becomes non-irritable in the course of 2 hours or more, but recovers immediately upon being replaced in Ringer's solution. It is remarkable that increase of oxygen tension may largely compensate for the reduction in salts. If oxygen be bubbled through the solutions the decrease of propagation-velocity may remain almost proportional to the decrease of electrical conductivity until only one-twentieth of the salts of Ringer's solution remain (*cf.* Table V)

Seventy-four series of experiments have been conducted upon propagation-velocities in varying mixtures of Ringer's solution and isotonic sugar solution. The order of the solutions used has been frequently altered to offset errors; while the time between the transfer of the muscle to a given solution and the taking of the record has always been long enough to allow the tissue to reach an equilibrium with the medium. Experiments 141, 142, and 143 (*cf.* Table III) are typical of the observations, and are selected with reference to the mixtures ranging from 30 per cent Ringer's solution to normal Ringer. The velocities and the conductivities are averaged and the ratios calculated in Table IV. In these experiments the mixtures were agitated continuously with a slow stream of air.

Experiments have been made on the velocity of the contraction-wave in solutions of still lower electrical conductivity, in which an increase of oxygen tension enables transmission to occur. Nine experiments in which oxygen was bubbled through the mixtures are recorded in Tables V and VI. In three of these experiments a mixture was employed containing only 5 per cent of the normal salt content of Ringer's solution. The muscle contracted and transmitted the contraction wave under these conditions; although in the same solutions containing oxygen at air tension no such behavior appears possible. In pure M/4 sugar solution saturated with oxygen these muscles lost irritability in slightly less than a half hour. Two of the experiments in the 0.05 Ringer's solution show a transmission rate very close to that calculated from the electrical conductivity.

TABLE III.

Contraction-Wave in Sartorius of Frog.

Record.	Solution.	Conductivity.	Velocity.	$\frac{v}{c} = k.$
Experiment 141; Wave Distance = 1.6 cm.; 20° C.				
B	Ringer	1.00	319	319
D	0.6R	0.58	174	300
G	0.4R	0.38	104	276
J	Ringer	1.00	319	319
Experiment 142; Wave Distance = 1.6 cm.; 20° C.				
A	Ringer	1.00	320	320
C	0.8R	0.81	258	319
D	0.5R	0.48	140	291
G	Ringer	1.00	320	320
J	0.3R	0.30	79	262
L	0.7R	0.69	215	312
N	Ringer	1.00	320	320
P	0.9R	0.92	288	313
R	Ringer	1.00	320	320
Experiment 143; Wave Distance = 1.7 cm.; 0 20° C.				
A	Ringer	1.00	306	306
G	0.3R	0.30	90	300
J	Ringer	1.00	306	306
M	0.9R	0.92	278	302
R	0.5R	0.48	127	265
S	Ringer	1.00	306	306
X	0.7R	0.69	213	309
Z	Ringer	1.00	306	306

TABLE IV.

Contraction-Wave in Sartorius of Frog.

(Average of Experiments 141, 142, 143.)

Solution.	Conductivity.	Velocity.	$\frac{v}{c} = k.$
Ringer	1.00	315	315
0.9R	0.92	283	308
0.8R	0.81	258	318
0.7R	0.69	214	310
0.6R	0.58	174	300
0.5R	0.48	134	279
0.4R	0.38	104	274
0.3R	0.30	85	283

In all of the solutions having a conductivity less than seven-tenths that of Ringer's solution the average ratio of velocity with respect to conductivity is higher than in Ringer's solution (*cf.* Table VI).

TABLE V.

Contraction-Wave in Sartorius of Frog.

(With Constant Stream of Oxygen.)

Solution.	Conductivity. (Ringer = 1.0)	Velocity per second.								
		Experiment No.								
		94	95	96	97	98	99	101	103	107
		cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
Ringer	1.00	295	310	315	312	300	320	300	306	315
0.9R	0.92	—	—	—	—	—	—	—	271	280
0.8R	0.81	—	246	—	250	—	—	245	—	—
0.7R	0.69	—	—	—	—	—	—	—	220	214
0.5R	0.48	150	—	160	—	144	—	147	—	155
0.4R	0.38	—	125	—	122	—	130	—	119	126
0.3R	0.30	100	—	—	—	—	—	95	—	—
0.2R	0.20	—	70	73	—	110	60	—	75	70
0.1R	0.11	40	—	45	—	—	35	—	—	—
0.05R	0.06	—	20	22	37	—	—	—	—	—
0.00R	0.0001	0	0	0	0	—	—	—	—	—

TABLE VI.

Contraction-Wave in Sartorius of Frog.

(Average Velocities of Table V.)

Solution.	Conductivity. (Ringer = 1.0)	Velocity per second.	$\frac{v}{c} = k.$
		cm.	
Ringer	1.00	308	308
0.9R	0.92	276	300
0.8R	0.81	247	304
0.7R	0.69	217	315
0.5R	0.48	151	314
0.4R	0.38	124	326
0.3R	0.30	97	323
0.2R	0.20	76	380
0.1R	0.11	40	458
0.05R	0.06	26	433

TABLE VII.

Contraction-Wave in Ventricle of Turtle.

Record.	Solution.	Conductivity (Ringer = 1.00).	Velocity per second.	$\frac{v}{c} = k$.
Experiment 152; Wave Distance = 2.0 cm.; 20°C.				
			cm.	
A	Ringer	1.00	7.6	7.6
D	0.5R	0.48	5.9	12.3
F	0.7R	0.69	6.4	9.2
H	Ringer	1.00	7.8	7.8
Experiment 154; Wave Distance = 1.6 cm.; 20°C.				
B	Ringer	1.00	8.8	8.8
E	0.8R	0.81	6.7	8.3
H	0.5R	0.48	4.0	8.3
L	0.4R	0.38	2.8	7.4
N	0.6R	0.58	4.8	8.3
P	Ringer	1.00	7.2	7.2
Experiment 155; Wave Distance = 3.0 cm.; 20°C.				
C	Ringer	1.00	9.1	9.1
E	0.4R	0.38	3.4	8.9
F	Ringer	1.00	7.5	7.5
Experiment 156; Wave Distance = 3.0 cm.; 20°C.				
C	Ringer	1.00	9.9	9.9
E	0.7R	0.69	6.0	8.7
H	0.9R	0.92	6.8	9.8
J	Ringer	1.00	8.8	8.8
M	0.5R	0.48	4.6	9.6
R	Ringer	1.00	6.5	6.5
Experiment 158; Wave Distance = 2.4 cm.; 20°C.				
B	Ringer	1.00	7.4	7.4
F	0.8R	0.81	7.1	8.7
G	0.6R	0.58	5.2	8.9
I	Ringer	1.00	6.9	6.9

Turtle.—Strips cut from the ventricle of the river terrapin were used in 1917 and 1921 with two different recording devices. The earlier observations were recorded by means of levers writing on smoked paper; in the later experiments the optical recording device

described above was used. In both sets of observations the initial speed is practically the same at about 20°C.; *viz.* about 7 cm. per second. Both the old and the newer readings agree also in respect to the rate and character of the changes undergone by the tissue under the experimental conditions. After a period of 6 hours in Ringer's solution the transmission-velocity begins to be irregular and the minimal stimulus in Ringer may fail to produce contraction. Upon changing the tissue end for end, or cutting off a little of the tissue on both ends, the regularity of response to the original minimal stimulus is restored and the transmission-velocity recovers. The strips of tissue selected for use have been from 3 to 5 mm. wide and from 3.5 to 4.5 cm. long. The observation points were between 0.6 and 3 cm. apart.

In 0.3 Ringer's solution strips of ventricle lose their irritability in the course of about 2 hours, and in pure isotonic sugar solution in about 50 minutes. Recovery of irritability upon replacement in normal Ringer's solution occurs in about 2 minutes. Little difference has been observed between the propagation-velocities in freshly prepared Ringer's solution and in older solutions through which air was bubbled.

The velocity of the contraction-wave is reduced in mixtures of Ringer with sugar solution; but in solutions of less than 0.6 Ringer the reduction of transmission-velocity is not proportional to the reduction in conductivity. Five experiments of the last series are recorded in Table VII.

DISCUSSION.

The data presented in this paper lend further support to the thesis that the electrical conductivity of the medium surrounding an activated protoplasmic system is in direct correlation with the rate of spread of the state of activation over the system. In skeletal muscle, the contraction-wave travels along the fibers at a velocity which is closely proportional to the electrical conductivity of the fluid in contact with it. A reduction, by means of dilution with an indifferent non-electrolyte solution, in the electrical conductivity of the normal balanced medium is followed by a corresponding reduction in the propagation-velocity of the contraction-wave. This parallelism

is closer in solutions of higher than in those of lower electrical conductivity. The ratio of velocity with respect to conductivity is nearly a constant with frog's muscle, and less constant with muscles of the turtle and *Limulus*.

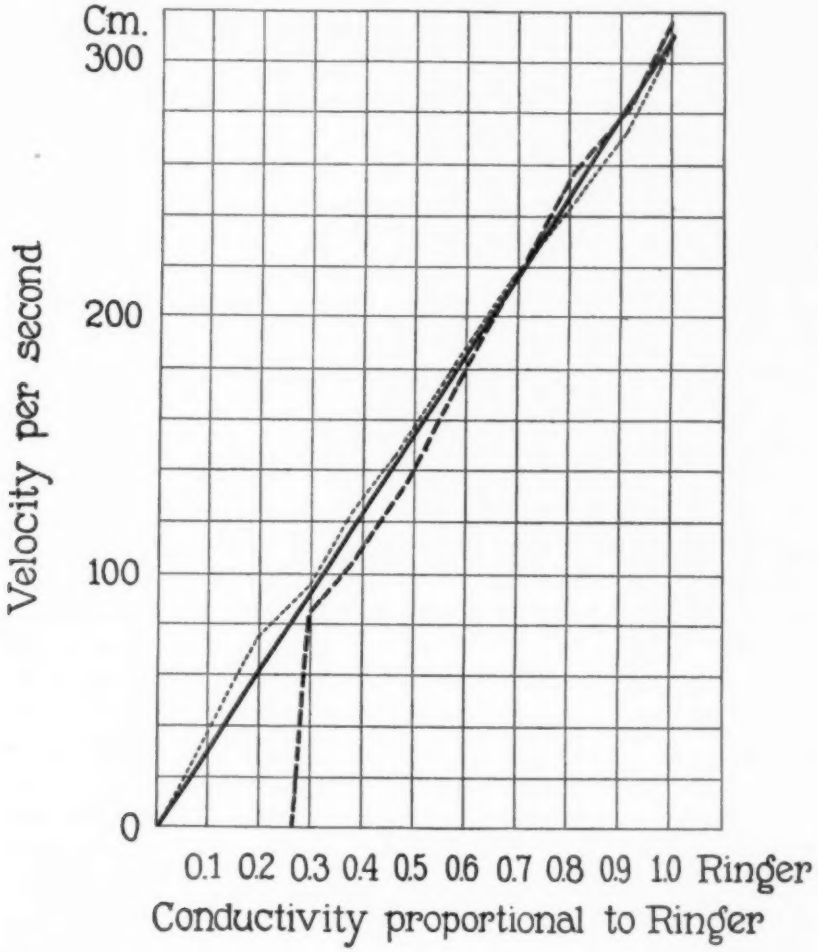

FIG. 1. Sartorius of frog in Ringer-sugar solution. Long dashes indicate aerated mixtures (Table IV); short dashes, oxygenated mixtures (Table VI).

In Fig. 1 the velocities of the contraction-wave in the sartorius of the frog are plotted against the conductivities of the solutions.

It will be seen that the dotted line runs closely parallel with the straight line which represents an ideal dependence of speed of conduction upon electrical conductivity. The dotted line connects the values secured when the muscle is immersed in a solution saturated with oxygen; while the dash line connects those obtained when the muscle is exposed to media containing oxygen at air tension.

The fact that in the presence of a stream of oxygen the transmission of the contraction-wave is possible even in solutions of very low conductivity is an indication that processes of oxidation are directly concerned in stimulation and in the propagation of the excitation state. If the primary change in stimulation is an alteration of the protoplasmic surface film, it would appear that in a medium of a given electrical conductivity a certain minimal concentration of oxygen is necessary for the completion of this surface reaction. Lillie has pointed out that such a relation of oxygen to a chemical reaction at the cell surface is suggestive of conditions similar to those of an electrolysis at an electrode. In this case it is "possible to reduce the current-strength through a wider range, and still have a high rate of decomposition at the electrode, if the concentration of the reacting substance is high, than if it is low."[14] In the present state of knowledge of the chemical conditions determining the formation of an active or "stimulated" region in living tissue, it is difficult to define clearly the rôle of oxygen in the stimulation process. In general it seems most probable that free oxygen is required in the return of the stimulated region to the resting state and in the propagation of the contraction-wave after the local stimulation has been aroused. If the analogy between activation in living tissues and in oxidisable metals bathed by electrolyte solutions, *e.g.* iron in nitric acid, is at all close, then the destruction of the surface film at any region during the rise of the activation-wave should depend upon a local reduction rather than upon an oxidation; apparently when the reduction has reached its maximum an oxidation process occurs, reforming the film and the passive or resting state is regained. In other words, the local excitation state is to be regarded as an effect resulting from the expenditure of energy in the stimulated region—

[14] Personal communication.

accompanying a reduction process; after the excited region has reached a maximum negativity (electrically negative with respect to adjacent regions) the reverse or oxidative process automatically follows, with the effect of reestablishing the passive or resting state. According to this hypothesis the free oxygen enters directly into only one part of the local stimulating process; but this oxidation must be repeated at each successive region during the propagation of the excitation state along the tissue.

If transmission in the living tissue is in fact dependent upon secondary electric stimulation of the resting region by the local bioelectric current between that region and the active region adjoining, there should be a direct proportionality between the electrical conductivity of the medium and the rate at which the state of activity spreads from region to region. The electrical conductivity of the first local stimulating circuit, other conditions being equal, determines the intensity of the current at any point in the circuit; and if the conductivity is uniform throughout the tissue and medium the rate of propagation will be uniform throughout the tissue. Differences in the electrical conductivity of the medium will be associated with differences in the rate of propagation because of the effect upon the intensity of the local bioelectric current traversing the tissue at any point adjacent to the active (electrically negative) area. The greater the conductivity of the medium, the greater will be the distance (from the already active region) at which the current traversing the resting tissue is sufficiently intense to effect the stimulating reaction. It is on the basis of such general considerations that Lillie believes we may provisionally disregard the special chemical nature of the local reaction (at the cell-medium boundary), on the ground that at present we know very little about it. Whatever the nature of the local stimulation process may happen to be, if it is initiated electrically by the current of the local bioelectric circuit it should spread from region to region at a rate proportional to the electrical conductivity of the circuit. This conductivity depends chiefly on the conductivity of the external medium.

In the preliminary work before any oxygen had been employed experimentally and when conditions were constant (as with air bubbled through the solutions or with fresh solutions) it seemed

possible that sugar might have some direct toxic action upon the tissues. Later considerations, however, make this doubtful. In the first place the parallelism between the electrical conductivity and propagation rate is closer with higher than with lower tensions of oxygen, although the sugar solutions are of the same concentration. In the second place if sugar acts toxically the toxic effect should increase proportionately to the increase in the length of exposure to the more concentrated sugar solutions. That this is not the case is indicated by the fact that the frog and *Limulus* tissues after immersion in pure sugar solution for periods up to 3 and 4 hours recover their irritability, upon return to Ringer's solution, just as rapidly and completely as when exposed to the pure sugar solution for periods just sufficient to abolish irritability. A further peculiarity in the action of sugar solutions, namely, the marked difference between the time required for loss of irritability in sugar solutions and for recovery of irritability on return to the normal conducting solution—the latter interval being many times shorter than the former—is not readily explained. It is beyond the aim of the present paper to discuss this problem, and it may be that the conditions must be more carefully investigated.

The author desires to thank Dr. Ralph S. Lillie for constant help and advice in the study of the present problem, and the Trustees of Clark University for the arrangements under which the research has been prosecuted.

DONNAN EQUILIBRIUM AND THE PHYSICAL PROPERTIES OF PROTEINS.

III. Viscosity.

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I. Volume of Solute and Viscosity.

The viscosity of freshly prepared gelatin solutions is affected in a similar way by the pH as are the electromotive forces, the osmotic pressure, and the swelling. We have been able to show that the influence of the pH on the E.M.F. can be accounted for quantitatively on the basis of the Donnan equilibrium,[1] and that with the exception of one or two minor deviations the same is true for the osmotic pressure.[2] Procter and Wilson's theory of swelling is also based on Donnan's theory of membrane equilibrium.[3] Fig. 1 is the expression of the influence of the pH on the viscosity of 0.5, 1, and 2 per cent freshly prepared gelatin chloride solutions at a temperature of 24°C. The abscissæ are the pH of the gelatin solution, while the ordinates are the relative viscosities of the gelatin solutions compared with that of water at the temperature of the experiment.

These curves are modified if the viscosity of the gelatin solution is not measured immediately, but only after the solution has been standing for some time. In this case the curve changes inasmuch as the viscosity rises everywhere but the more rapidly, the nearer the pH is to that of the isoelectric point. In this case the curves representing the influence of pH on the viscosity of gelatin solution no longer resemble the curves representing the influence of the pH

[1] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 577, 667.

[2] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 691.

[3] Procter, H. R., *J. Chem. Soc.*, 1914, cv, 313. Procter, H. R., and Wilson, J. A., *J. Chem. Soc.*, 1916, cix, 307.

on the osmotic pressure and swelling. In the literature, however, it is usually stated that the influence of acid on viscosity resembles that of acids on osmotic pressure and swelling, and the question arises whether or not the theory of the Donnan equilibrium can be applied to the explanation of this type of viscosity curves found when freshly prepared gelatin solutions are used (Fig. 1).

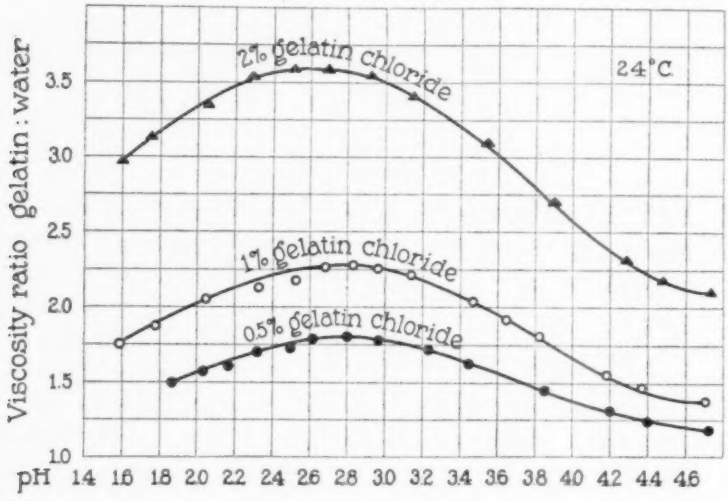

FIG. 1. Influence of pH on viscosity of freshly prepared gelatin chloride solutions.

Several formulæ exist for the calculation of the influence of a solute on the viscosity of a solvent. The first one (1) was derived by Einstein[4]

$$\eta = \eta_0 (1 + 2.5 \varphi) \qquad (1)$$

where η_0 is the viscosity of the pure solvent at the temperature of the experiment, η the viscosity of the solution, and φ the fraction of the volume occupied by the solute in the solution. This formula holds only when φ is very small and when the particles of the solute are spherical and large compared with the molecules of the solvent.

[4] Einstein, A., *Ann. Physik*, 1906, xix, 289; 1911, xxxiv, 591.

Hatschek,[5] Smoluchowski,[6] Hess,[7] and Arrhenius[8] have modified Einstein's formula so as to make it valid for any concentration. Arrhenius replaces the linear by a logarithmic formula

$$\text{Log } \eta - \text{Log } \eta_0 = \theta\varphi \qquad (2)$$

where φ is again the fraction of volume occupied by the solute in the solution and θ a constant, while η and η_0 have the same significance as in Einstein's formula.

All the formulæ agree in one point, namely that the fraction of the volume occupied by the solute in the solution is the main variable upon which the relative viscosity of a solution depends. It has been pointed out by Odén[9] and others that in addition to the relative volume occupied by suspended particles the average size of the individual granules in a suspension plays also a rôle in viscosity. According to these theories of viscosity, it should be possible to correlate the characteristic influence of the hydrogen ion concentration upon the viscosity of gelatin solutions with a variation in the relative volume or the average size of the gelatin particles in solution, since the mass of gelatin in solution remains the same in these experiments.

The measurement of the viscosity is in our experiments the time of outflow of the solution through a capillary tube and the method of the experiments (already described in a previous paper) was briefly as follows. To 50 cc. of a 2 per cent solution of isoelectric gelatin is added the desired acid, *e.g.*, HCl, in sufficient quantity and then the volume is raised to 100 cc. by the addition of enough distilled water. This 1 per cent solution of originally isoelectric gelatin is rapidly heated to 45°C., kept at that temperature for 1 minute, and then rapidly cooled to 24°C. (or any other desired temperature). The viscosity is measured immediately after the solution was cooled to 24°C., since on standing the viscosity increases unequally at different pH. The measurements were all made by determining the time of outflow through a capillary tube. The time of

[5] Hatschek, E., *Kolloid Z.*, 1913, xii, 238; 1920, xxvii, 163.

[6] Smoluchowski, M. v., *Kolloid Z.*, 1916, xviii, 190.

[7] Hess, W. R., *Kolloid Z.*, 1920, xxvii, 1, 154.

[8] Arrhenius, S., *Meddelanden from K. Vetenskapsakademiens Nobelinstitut*, 1917, iii, No. 21.

[9] Odén, S., *Nova acta regiae Societatis Scientiarum Upsaliensis*, 1913, iii, No. 4.

outflow for pure water was 56 seconds at 24°C. The pH of the solutions was determined with the aid of the potentiometer.

The reader will notice (Fig. 1) that the relative viscosity of a gelatin solution is a minimum at the isoelectric point (pH = 4.7), that it rises with a rise in the hydrogen ion concentration until it becomes a maximum at pH of about 2.7, and that it drops again with a further rise in the hydrogen ion concentration. The question is how to explain the apparent changes in the relative volume of the gelatin in solution which, according to the theory, must be the main cause of the variation of the viscosity with the pH.

A change in the ratio of volume of gelatin to volume of water is only possible if water is added to the gelatin. Pauli[10] suggested that the ionized particle of protein is surrounded by a shell of water which is lacking in the non-ionized molecule. The volume of the protein ions in solution is increased by this jacket of water. Since the gelatin in solution is practically non-ionized at the isoelectric point, the relative volume of the gelatin in solution is a minimum at this point, while when we add an acid, *e.g.*, HCl, gelatin chloride is formed, which, like all salts, ionizes readily. On this basis we can understand why the viscosity should increase with an increase of the hydrogen ion concentration of the gelatin solution; since with this increase in the hydrogen ion concentration, the concentration of hydrated gelatin ions and hence the volume of the gelatin particles should also increase. The work of Lorenz,[11] Born,[12] and others casts, however, a doubt on the assumption of a general hydration of polyatomic ions. We shall see presently that there are still other facts which show that the mere ionization and consequent hydration of the individual protein ions cannot well be the cause of the influence of the pH on the relative viscosity of gelatin solutions.

II. The Influence of the Hydrogen Ion Concentration on the Viscosity of Solutions of Amino-Acids and of Crystalline Egg Albumin.

If hydration of the individual protein ions were the cause of the variation of the viscosity of gelatin solutions, a variation of the hy-

[10] Pauli, W., *Kolloid Z.*, 1913, xii, 222.
[11] Lorenz, R., *Z. Elektrochem.*, 1920, xxvi, 424.
[12] Born, M., *Z. Elektrochem.*, 1920, xxvi, 401.

drogen ion concentration should have a similar influence on the viscosity of solutions of simple amino-acids, like glycocoll and alanine, as it has on the viscosity of gelatin solution. 5 per cent solutions of glycocoll and alanine were brought to different pH, from 5.0 to 2.0 and below, by the addition of HCl. The variation of the pH of 5 per cent solutions of these two amino-acids between the limits of 5.0 and 1.16 had no measurable influence on the viscosity of the solution. This cast a serious doubt on the assumption that the variations in the curve of the viscosity of gelatin, as expressed in Fig. 1, were caused by variations in the hydration of the individual gelatin ions.[13]

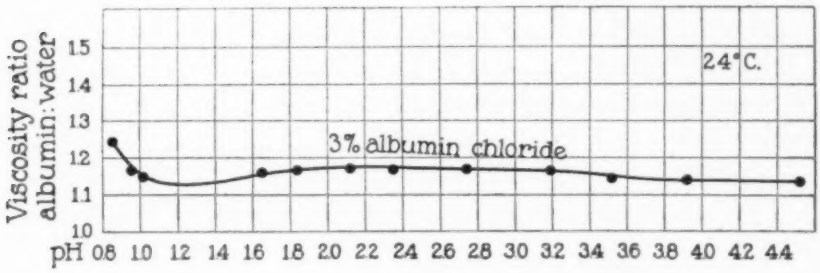

FIG. 2. Showing that solutions of crystalline egg albumin have a low viscosity in comparison with gelatin solutions, and that the pH has little influence on the viscosity of solutions of crystalline egg albumin at pH over 1.0 and at ordinary temperature.

This doubt was increased by experiments on the influence of pH on the viscosity of crystalline egg albumin which gave also a practically negative result. Fig. 2 gives such an experiment with 3 per cent originally isoelectric albumin brought to different pH through the addition of HCl. The ordinates are the viscosity ratios of albumin solution over water, drawn on a larger scale than those in Fig. 1, and the abscissæ are the pH of the solution. It is obvious that the pH has only a very slight if any influence on the viscosity of solutions of crystalline egg albumin between pH 4.6 and pH 1.0. With a further lowering of pH the viscosity suddenly rises, a fact to which we shall return later. It is also obvious that the viscosity

[13] These experiments were carried out by Dr. Elizabeth Brakeley.

ratio, protein solution: water, is considerably smaller in the case of albumin solutions than in the case of gelatin solutions.

The method of the experiments was as follows. 50 cc. of a 6 per cent solution of isoelectric crystalline egg albumin were mixed with 50 cc. of HCl solution of different concentration and the pH measured. The solution was rapidly brought to a temperature of 24°C. and the viscosity was measured immediately at that temperature.

The question then arises, why do amino-acids and at least one protein, namely crystalline egg albumin, behave so differently from gelatin in regard to the influence of the pH on the viscosity? As long as we assume that the influence of the hydrogen ion concentration on the viscosity of gelatin-acid salt solution is due to the hydration of the individual protein ions this difference is incomprehensible since the amino-acids as well as crystalline egg albumin should in this case show the same influence of ionization on hydration as the gelatin.

The puzzle becomes still greater if we take into consideration the fact that the osmotic pressure of solutions of crystalline egg albumin is affected in the same way by the hydrogen ion concentration as is the osmotic pressure of gelatin solutions. Why then do these two proteins behave so differently as regards the influence of the pH on their viscosity?

To answer this question we are forced to the conclusion that gelatin in solution must possess a way of increasing its volume which is lacking in the case of solutions of crystalline egg albumin (at least at ordinary temperature and at a pH above 1.0). This difference seems to be connected with a difference in the ability to form a gel. Solutions of isoelectric crystalline egg albumin of a high concentration can be kept for many months at a temperature just above the freezing point without setting to a jelly or without even showing an increase in viscosity; while solutions of isoelectric gelatin of even a low concentration show a rapid increase in viscosity and may set to a jelly under the same conditions of temperature and pH which do not alter the viscosity of egg albumin. Moreover, crystalline egg albumin has a very low viscosity compared with that of the same mass of gelatin in solution.

When, however, the pH of a 3 per cent solution of crystalline egg albumin falls to 0.85 or below, the solution can set to a gel and in that case its viscosity rises to the same order of magnitude as the viscosity of gelatin solutions except that an increase in temperature has the opposite effect as in the case of gelatin solutions. This is illustrated by Figs. 3 and 4. Both figures show the influence of time on the

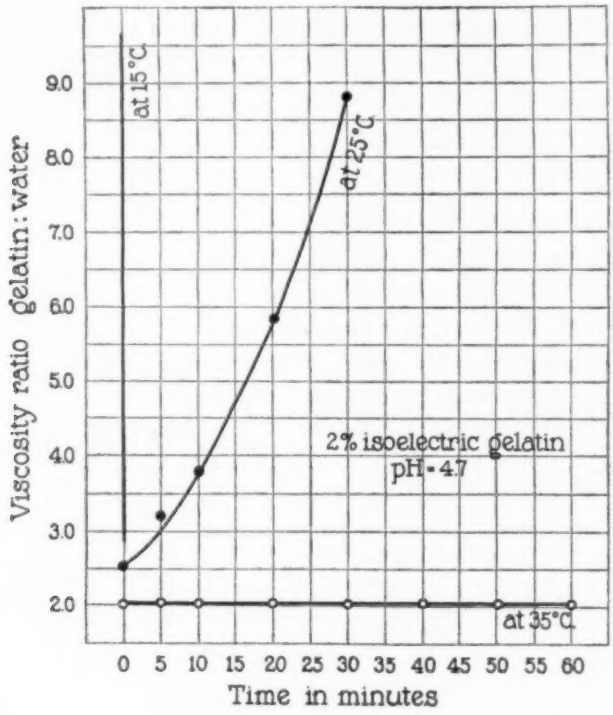

FIG. 3. Influence of time on viscosity of isoelectric gelatin solutions at different temperatures.

viscosity ratio of solutions of gelatin or albumin to that of pure water. Fig. 3 shows that the viscosity of a 2 per cent solution of isoelectric gelatin rises rapidly at 15°C., more slowly at 25°C., and quite slowly at 35°C.

Fig. 4 gives the influence of time on the viscosity of 3 per cent solutions of albumin chloride at pH 0.85 where the solutions are

opalescent and have a tendency to set to a gel. The reader will notice that at that pH viscosity of solutions of albumin chloride behaves like the viscosity of gelatin solutions, inasmuch as the viscosity of albumin chloride solutions also rises with time as soon as

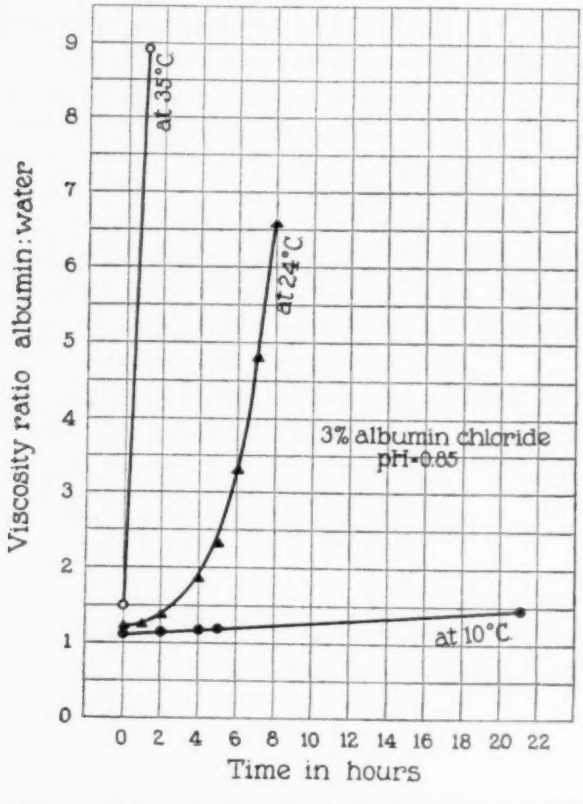

FIG. 4. Influence of time on viscosity of solutions of crystalline egg albumin of pH 0.85 at different temperatures. At this pH the albumin chloride solutions have a tendency to set to a jelly and have the same high order of viscosity as the gelatin solutions.

the solution acquires the property of setting to a gel, and that the order of magnitude of the viscosity of albumin chloride solutions capable of setting to a gel no longer differs from that of gelatin solution. The only difference is that of the influence of temperature on the viscosity which is the reverse in the case of the two proteins.

High temperature favors jelly formation in the case of egg albumin and retards it in the case of gelatin.

These observations corroborate the suspicion that the high order of magnitude of viscosity of gelatin solutions may be in some way connected with the tendency of this protein to set to a gel.

We do not yet know how the tendency of a protein solution to form a gel can account for the following two facts: first, that this tendency is accompanied by a rise in viscosity, and second, that the pH influences the viscosity in a way suggestive of the Donnan equilibrium. One possible answer to this question might be that the formation of a continuous gel by a protein solution may be preceded by the formation of a number of submicroscopic particles of gel, each occluding a considerable amount of water. This occlusion of water would cause a considerable increase in the volume of the mass of gelatin and this could account for the rise in viscosity with the tendency to form a gel. The Donnan equilibrium would regulate the quantity of water occluded by each particle and this would account for the influence of pH. The idea of such a possibility gave rise to the following experiments on the viscosity of suspensions of powdered gelatin in water.

III. Influence of Volume of Powdered Gelatin on Viscosity.

When we suspend finely powdered gelatin in water of a sufficiently low temperature, and measure the viscosity of such suspensions we find that they may have even a higher viscosity than gelatin solutions of the same concentration of gelatin and that the pH influences the viscosity of the suspension in the same characteristic way as that expressed in the curves of Fig. 1.

0.5 gm. of Cooper's powdered commercial gelatin of a pH of about 6.0 was added to 100 cc. of water containing varying amounts of HCl. The particles had uniform size (going through Sieve 100 but not through Sieve 120), but their shape was extremely irregular. They were left in the solution several hours at 20°C., and then their time of outflow through a capillary tube was ascertained at 20°C. The time of outflow of water through the viscometer at this temperature was 24 seconds. It was essential to stir the suspension thoroughly

before sucking it into the viscometer since the gelatin particles sink rapidly to the bottom of the dish.

After the viscosity measurements were taken, the suspension was put on a filter of cotton wool and the supernatant water allowed to drain off. By measuring the volume of the filtrate and deducting this from the original volume of the suspension (which was in all cases 100 cc.), the volume of the gelatin (with some error) was obtained. Then the gelatin was melted, and the pH of the melted mass of gelatin as well as of the filtrate was determined potentiometrically. Fig. 5 gives the result of such an experiment. The lower

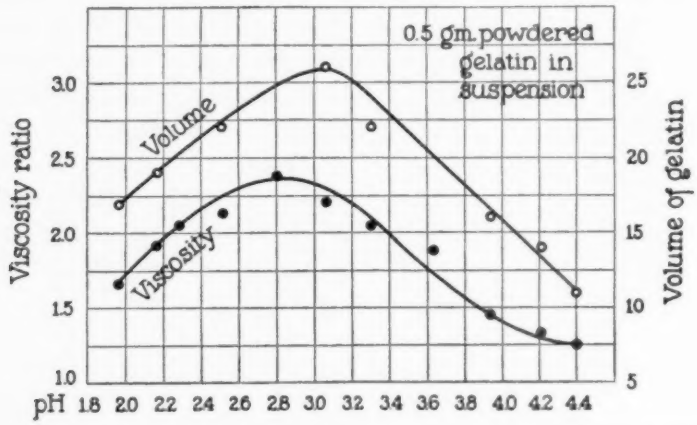

FIG. 5. Showing that the influence of pH on viscosity of 0.5 per cent suspensions of powdered gelatin in water is similar to the influence of pH on viscosity of gelatin solutions, and that the volume occupied by the particles in the suspension varies in a similar way as the viscosity. Temperature 20°C.

curve shows the influence of the pH (of the gelatin) on the viscosity, and the upper curve the influence of the pH on the volume of the gelatin. The two curves are similar.

Moreover, the viscosity values for the suspensions of 0.5 gm. of powdered particles in water are greater than the viscosity values of a 0.5 per cent solution of freshly liquefied gelatin for equal pH.[14] The highest viscosity ratio of the 0.5 per cent gelatin solution was about 1.75 while the highest viscosity ratio for the 0.5 per cent suspension of gelatin was about 2.4 (Fig. 5).

[14] The viscosity of the solution increases on standing.

These experiments prove, first, that a suspension of powdered gelatin in water shows the same variation in viscosity with the variation of the hydrogen ion concentration as does a solution of freshly prepared gelatin; and, second, that the relative volume of the suspended particles varies in a similar way as the viscosity (Fig. 5). In this case there is little doubt that the variations in the volume of the suspended particles of gelatin under the influence of the pH are due to the existence of a Donnan equilibrium between the particles and the surrounding water, since we have already shown in a former publication that there exists a difference in the pH of the solid particles of powdered gelatin and the supernatant water and this fact was further corroborated in these experiments (Table I).

TABLE I.

Donnan Equilibrium Between Suspended Particles of Gelatin and Supernatant Water After 20 Hours.

0.5 gm. of Gelatin Suspension in 100 cc. H_2O Containing Various Amounts of HCl at Temperature of 20°C.

pH of gelatin particles	4.79	4.62	4.33	4.17	3.93	3.60	3.26	3.02	2.68	2.39	2.16	2.07	1.80
pH of supernatant water	4.74	4.30	3.95	3.76	3.55	3.21	2.95	2.77	2.56	2.31	2.10	2.02	1.75

The point which is of importance is the question of the applicability of Einstein's formula to these experiments

$$\frac{\eta}{\eta_0} = 1 + 2.5\varphi$$

The fact that the shape of the suspended particles of gelatin is very irregular and that the average size of the individual particles plays also a rôle must warn us not to expect too strict an applicability of the formula in our case; and we may expect to obtain slightly different values than 2.5 for the constant. Since we can measure $\frac{\eta}{\eta_0}$ as well as φ directly in our experiments, we may write Einstein's equation in the form

$$\frac{\eta}{\eta_0} - 1 = c\varphi$$

and may try to calculate the value of $c = \frac{\frac{\eta}{\eta_0} - 1}{\varphi}$ from our observations. Since φ is the ratio of the volume of the gelatin to the volume of the solution and the latter is 100 cc., we have to multiply the value $\frac{\frac{\eta}{\eta_0} - 1}{\text{volume of gelatin}}$ by 100 to obtain c.

Table II shows the results of such a calculation.

TABLE II.

pH of gelatin.	$\frac{\eta}{\eta_0}$	Volume of gelatin.	c
		cc.	
4.80	1.070	4.5	1.5
4.40	1.250	8.0	3.1
4.21	1.345	11.0	3.1
3.94	1.515	16.0	3.2
3.63	1.845	18.0	4.2
3.30	2.120	21.0	5.3
2.80	2.340	22.5	6.0
2.51	2.150	19.5	5.9
2.28	2.080	18.0	6.0
2.16	1.865	16.0	5.4
1.96	1.726	16.0	4.5

Considering the fact that the gelatin particles are not perfect spheres, as Einstein's theory demands, and considering the further fact that the measurements of the volume of gelatin are crude, it is surprising that where the volume of the gelatin is small the constant c is so near that expected on the basis of Einstein's formula, namely 3.1 instead of 2.5. Larger values are found (from 4.2 to 6.0) when the swelling of the gelatin particles becomes too large to permit the strict application of Einstein's formula. In all probability a second variable enters in this case, namely the large size of the individual granules. We shall see in a later paper that in the case of suspensions of gelatin particles the viscosity is not only a function of the relative volume of the suspended particles but also of their size, increasing (for the size used in our experiments) with the size. This might account for the fact that in Table II the value of c varies with the size of the particles.

If we consider all these complicating circumstances there can be little doubt left that the influence of pH on the viscosity of suspensions of particles of gelatin is mainly due to the change in volume of these particles under the influence of the pH and that this change of volume finds its explanation in the Donnan equilibrium between the particles and the surrounding liquid.

It is well known, and it has been discussed in preceding papers,[15] that the viscosity of a gelatin chloride solution, *e.g.*, of pH 3.0, is

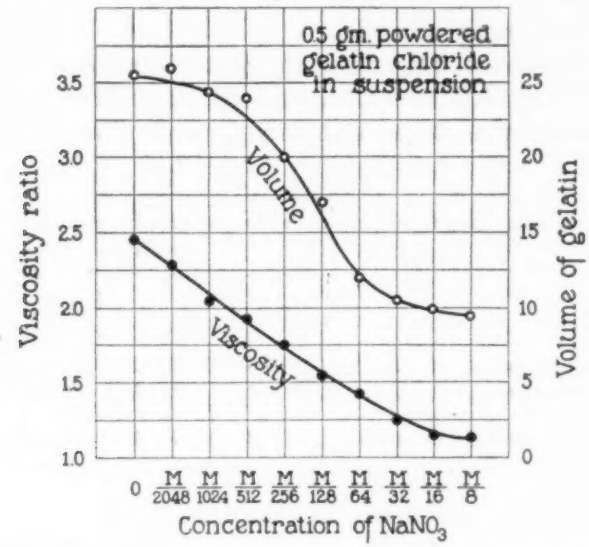

FIG. 6. Showing depressing influence of neutral salts on viscosity of suspensions of powdered gelatin in water and on the volume occupied by the gelatin particles in the suspension.

lowered when neutral salts are added and the pH kept constant. The same is true for the viscosity of suspensions of powdered gelatin. Doses of 0.5 gm. of powdered gelatin of pH 6.0, going through Sieve 100 but not through Sieve 120, were put each into 100 cc. of water containing 6 cc. of 0.1 N HCl, and different quantities of $NaNO_3$, so that the concentration of the salt varied in the different solutions from M/8 to M/2048. One solution contained no salt. The pH of the

[15] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 391.

gelatin varied in the neighborhood of 3.0; the temperature was 20°C. After 2½ hours, when the Donnan equilibrium between the particles and the surrounding solution was supposed to be established, the viscosity of each suspension was measured at 20°C. and the volume occupied by the suspended particles of gelatin was ascertained in the manner described. It was found that the addition of salt diminished the relative volume of the gelatin particles and the viscosity in a similar way (Fig. 6). The observed volume of the solutions containing little or no salt was probably a little too great on account of incomplete filtration.

The measurement of the pH of the gelatin solution and the outside solution showed that the addition of salt diminished the difference between the two, as Donnan's theory demands (Table III).

TABLE III.

	Concentration of $NaNO_3$.								
	0	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16
pH of gelatin particles	3.04	3.04	3.03	3.02	3.00	3.02	2.97	2.94	2.85
pH of supernatant liquid	2.74	2.76	2.76	2.76	2.77	2.80	2.78	2.77	2.70
Difference, pH inside minus pH outside	0.30	0.28	0.27	0.26	0.23	0.22	0.19	0.17	0.15

SUMMARY AND CONCLUSION.

1. Gelatin solutions have a high viscosity which in the case of freshly prepared solutions varies under the influence of the hydrogen ion concentration in a similar way as the swelling, the osmotic pressure, and the electromotive forces. Solutions of crystalline egg albumin have under the same conditions a comparatively low viscosity which is practically independent of the pH (above 1.0). This difference in the viscosities of solutions of the two proteins seems to be connected with the fact that solutions of gelatin have a tendency to set to a jelly while solutions of crystalline egg albumin show no such tendency at low temperature and pH above 1.0.

2. The formulæ for viscosity demand that the difference in the order of magnitude of the viscosity of the two proteins should cor-

respond to a difference in the relative volume occupied by equal masses of the two proteins in the same volume of solution. It is generally assumed that these variations of volume of dissolved proteins are due to the hydration of the isolated protein ions, but if this view were correct the influence of pH on viscosity should be the same in the case of solutions of gelatin, of amino-acids, and of crystalline egg albumin, which, however, is not true.

3. Suspensions of powdered gelatin in water were prepared and it was found, first, that the viscosity of these suspensions is a little higher than that of gelatin solutions of the same concentration, second, that the pH influences the viscosity of these suspensions similarly as the viscosity of freshly prepared gelatin solutions, and third, that the volume occupied by the gelatin in the suspension varies similarly as the viscosity which agrees with the theories of viscosity. It is shown that this influence of the pH on the volume occupied by the gelatin granules in suspension is due to the existence of a Donnan equilibrium between the granules and the surrounding solution.

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